[redacted]

STIC-ILL

mic
QH442.G43

Fr m: Sullivan, Daniel
Sent: Monday, October 21, 2002 12:26 PM
T : STIC-ILL
Subject: Request

Please send the following:

ACCESSION NUMBER: 1990:155203 BIOSIS
SOURCE: GENE (AMST), (1989) 84 (2), 429-438

Gene 1988 Mar 31;63(2):321-30

Thank you.

Daniel M. Sullivan
Examiner AU 1636
Room: 12D12
Mail Box: 11E12
Tel: 703-305-4448

09957031

103

[redacted]

STIC-ILL

MIC
QH442.G43

Fr m: Sullivan, Daniel
Sent: Monday, October 21, 2002 12:26 PM
To: STIC-ILL
Subject: Request

Please send the following:

ACCESSION NUMBER: 1990:155203 BIOSIS
SOURCE: GENE (AMST), (1989) 84 (2), 429-438

Gene 1988 Mar 31;63(2):321-30

Thank you.

Daniel M. Sullivan
Examiner AU 1636
Room: 12D12
Mail Box: 11E12
Tel: 703-305-4448

09957031

Poss 103
combine C
Encoding of transducer
C kp prot

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1636DXS

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

********** Welcome to STN International **********

NEWS 1 Web Page URLs for STN Seminar Schedule - N. America
NEWS 2 Apr 08 "Ask CAS" for self-help around the clock
NEWS 3 Apr 09 BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 4 Apr 09 ZDB will be removed from STN
NEWS 5 Apr 19 US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 6 Apr 22 Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 7 Apr 22 BIOSIS Gene Names now available in TOXCENTER
NEWS 8 Apr 22 Federal Research in Progress (FEDRIP) now available
NEWS 9 Jun 03 New e-mail delivery for search results now available
NEWS 10 Jun 10 MEDLINE Reload
NEWS 11 Jun 10 PCTFULL has been reloaded
NEWS 12 Jul 02 FOREGE no longer contains STANDARDS file segment
NEWS 13 Jul 22 USAN to be reloaded July 28, 2002; saved answer sets no longer valid
NEWS 14 Jul 29 Enhanced polymer searching in REGISTRY
NEWS 15 Jul 30 NETFIRST to be removed from STN
NEWS 16 Aug 08 CANCERLIT reload
NEWS 17 Aug 08 PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 18 Aug 08 NTIS has been reloaded and enhanced
NEWS 19 Aug 19 Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 20 Aug 19 IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21 Aug 19 The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22 Aug 26 Sequence searching in REGISTRY enhanced
NEWS 23 Sep 03 JAPIO has been reloaded and enhanced
NEWS 24 Sep 16 Experimental properties added to the REGISTRY file
NEWS 25 Sep 16 Indexing added to some pre-1967 records in CA/CAPLUS
NEWS 26 Sep 16 CA Section Thesaurus available in CAPLUS and CA
NEWS 27 Oct 01 CASREACT Enriched with Reactions from 1907 to 1985
NEWS 28 Oct 21 EVENTLINE has been reloaded

NEWS EXPRESS October 14 CURRENT WINDOWS VERSION IS V6.01,
CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP),
AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002
NEWS HOURS STN Operating Hours Plus Help Desk Availability
NEWS INTER General Internet Information
NEWS LOGIN Welcome Banner and News Items
NEWS PHONE Direct Dial and Telecommunication Network Access to STN
NEWS WWW CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

********** STN Columbus **********

FILE 'HOME' ENTERED AT 11:48:35 ON 21 OCT 2002

=> file medline biosis caplus
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 0.21 0.21

FILE 'MEDLINE' ENTERED AT 11:48:46 ON 21 OCT 2002

FILE 'BIOSIS' ENTERED AT 11:48:46 ON 21 OCT 2002
COPYRIGHT (C) 2002 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'CAPLUS' ENTERED AT 11:48:46 ON 21 OCT 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

=> s cochleate
L1 127 COCHLEATE

=> s multilamellar lipid
L2 230 MULTILAMELLAR LIPID

=> s l1 or l2
L3 357 L1 OR L2

=> s aav or adeno associated
L4 6610 AAV OR ADENO ASSOCIATED

=> s l3 and l4
L5 1 L3 AND L4

=> d ti so

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
TI Integrative protein-DNA **cochleate** formulations and methods for transforming cells
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2

=> d ibib ab

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:420934 CAPLUS
DOCUMENT NUMBER: 133:48870
TITLE: Integrative protein-DNA **cochleate** formulations and methods for transforming cells
INVENTOR(S): Margolis, David; Gould-fogerite, Susan; Mannino, Raphael James
PATENT ASSIGNEE(S): University of Maryland, USA; University of Medicine and Denistry of New Jersey
SOURCE: PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

[redacted]

PATENT NO. KIND DATE APPLICATION NO. DATE
---------- ---- -------- ---------- --------
WO 2000035421 A2 20000622 WO 1999-US29446 19991213
WO 2000035421 A3 20001109
W: AU, CA, JP
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
US 6340591 B1 20020122 US 1998-210578 19981214
EP 1140023 A2 20011010 EP 1999-966144 19991213
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
US 2002034822 A1 20020321 US 2001-957031 20010921
PRIORITY APPLN. INFO.: US 1998-210578 A 19981214
WO 1999-US29446 W 19991213
AB An integrative DNA vector and one or more viral proteins having affinity
for DNA are packaged in **cochleate** ppts. The integrative DNA vector contains one or more therapeutic nucleotide sequences that are preferably positioned between DNA substrates for the proteins. Upon
contact with a lipid bilayer of a target cell, the **cochleate** vector structure delivers one or more of the therapeutic nucleotide sequences and one or more proteins to the interior of the target cell. Upon entry into the cell, the proteins facilitate the integration of the therapeutic nucleotide sequence into the genome of the host cell.

=> d his

(FILE 'HOME' ENTERED AT 11:48:35 ON 21 OCT 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 11:48:46 ON 21 OCT 2002
L1 127 S COCHLEATE
L2 230 S MULTILAMELLAR LIPID
L3 357 S L1 OR L2
L4 6610 S AAV OR ADENO ASSOCIATED
L5 1 S L3 AND L4

=> rep!
REP! IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> rep
REP IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s rep!
L6 5096 REP!

=>]s l6 and l3
]S IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s l6 and l3
L7 0 L6 AND L3

=> s rep
L8 8791 REP

=> s l8 and l3
L9 1 L8 AND L3

=> d ti so

L9 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
TI Integrative protein-DNA **cochleate** formulations and methods for transforming cells
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2

=> s recombinase
L10 6979 RECOMBINASE

=> s l3 and l10
L11 0 L3 AND L10

=> s recombin?
L12 694865 RECOMBIN?

=> s l3 and l12
L13 4 L3 AND L12

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14 4 DUP REM L13 (0 DUPLICATES REMOVED)

=> d ti so 1-4

L14 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2002 ACS
TI Structural and functional characterization of liposomal **recombinant** hepatitis B vaccine
SO Journal of Liposome Research (1996), 6(2), 289-304
CODEN: JLREE7; ISSN: 0898-2104

L14 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2002 ACS
TI Liposome preparation and material encapsulation method
SO PCT Int. Appl., 34 pp.
CODEN: PIXXD2

L14 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI CHIMERASOME-MEDIATED GENE TRANSFER IN-VITRO AND IN-VIVO.
SO GENE (AMST), (1989) 84 (2), 429-438.
CODEN: GENED6. ISSN: 0378-1119.

L14 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2002 ACS
TI Diastereoselectivity in radical pair **recombination** in lipid bilayer
SO J. Am. Chem. Soc. (1984), 106(24), 7652-3
CODEN: JACSAT; ISSN: 0002-7863

=> d ibib ab 2,3

L14 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:698980 CAPLUS
DOCUMENT NUMBER: 123:93303
TITLE: Liposome preparation and material encapsulation method
INVENTOR(S): Collins, David Sammuel
PATENT ASSIGNEE(S): Amgen Inc., USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO. KIND DATE APPLICATION NO. DATE
---------- ---- -------- ---------- --------
WO 9512387 A1 19950511 WO 1994-US12350 19941103
W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
CA 2153251 AA 19950511 CA 1994-2153251 19941103
CA 2153251 C 19980901

AU 9481273 A1 19950523 AU 1994-81273 19941103
AU 683957 B2 19971127
EP 678017 A1 19951025 EP 1995-900452 19941103
EP 678017 B1 19980401
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE
JP 08505882 T2 19960625 JP 1994-513303 19941103
AT 164515 E 19980415 AT 1995-900452 19941103
ES 2115343 T3 19980616 ES 1995-900452 19941103
US 5567433 A 19961022 US 1995-381613 19950130
US 6355267 B1 20020312 US 1997-868019 19970603
PRIORITY APPLN. INFO.: US 1993-148099 A 19931105
WO 1994-US12350 W 19941103
US 1995-394056 B1 19950224

AB The present invention relates to a method of producing liposomes useful for encapsulating and delivering a wide variety of biol. active materials. The invention provides liposomes and a prodn. method which is simple, feasible and inexpensive for the large-scale com. manufg. of liposomes and encapsulated materials. The method involves the formation of a liposome dispersion in the absence of an org. solvent or detergent, one or several cycles of freezing and thawing the liposomes, and dehydration of the liposome dispersion to form a lipid powder. When desired, the lipid powder is hydrated in the presence of the biol. active material whereby the material is encapsulated in reconstituted liposomes. The method can also include combining the liposome dispersion with a bulking agent prior to the dehydration and formation of the lipid powder. The addn. of the bulking agent facilitates the handling of the lipid powder as well as its rapid dispersal upon hydration. Chloroform solns. of dimyristoylphosphatidylcholine, distearoylphosphatidylcholine, and cholesterol were combined in a ratio of 1:4:5, resp., and the mixt. was dried and desiccated to form a lipid film. The lipid was then hydrated in presence of **recombinant** human granulocyte colony stimulating factor at 60.degree. and the sample was microfluidized to obtain liposomes.

L14 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1990:155203 BIOSIS
DOCUMENT NUMBER: BA89:82621
TITLE: CHIMERASOME-MEDIATED GENE TRANSFER IN-VITRO AND IN-VIVO.
AUTHOR(S): GOULD-FOGERITE S; MAZURKIEWICZ J E; RASKA K JR; VOELKERDING K; LEHMAN J M; MANNINO R J
CORPORATE SOURCE: DEP. MICROBIOL. IMMUNOL., A-68, NEIL HELLMAN MED. RES. BUILD., ALBANY MED. COLL., 47 NEW SCOTLAND AVE., ALBANY, N.Y. 12208, USA.
SOURCE: GENE (AMST), (1989) 84 (2), 429-438. CODEN: GENED6. ISSN: 0378-1119.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Proteoliposome delivery vesicles can be prepared by the protein-**cochleate** method [Gould-Fogerite and Mannino, Anal. Biochem. 148 (1985) 15-25; Mannino and Gould-Fogerite, Biotechniques 6 (1988) 682-690]. Proteins which mediate the entry of enveloped viruses into cells are integrated in the lipid bilayer, and materials are encapsulated at high efficiency within the aqueous interior of these vesicles. We describe proteoliposome-mediated delivery of proteins and drugs into entire populations of cells in culture. Material can be delivered gradually by Sendai-virus-glycoprotein-containing proteoliposomes. Alternatively, synchronous delivery to a population can be achieved by exposing cell-bound influenza glycoprotein vesicles briefly to low pH buffer. When DNA is encapsulated, chimeric proteoliposome gene-transfer vesicles (chimerasomes), which mediate high-efficiency gene transfer in vitro and in vivo, are produced. Stable expression of bovine papilloma virus-based plasmid in tissue-culture cells, at 100,000 times greater efficiency than Ca.cntdot.phosphate precipitation of DNA, with respect to the quantity of DNA used, has been achieved. Stable gene transfer and expression in mice has been obtained by subcutaneous injection of chimerasomes containing a plasmid expressing the early region of polyoma virus. In one experimental group, 50% of the mice developed tumors which were shown to express polyoma virus early proteins and contain the transferred DNA. This is the first report of stable gene transfer in animals mediated by aa liposome- or proteoliposome-based system.

=> d his

(FILE 'HOME' ENTERED AT 11:48:35 ON 21 OCT 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 11:48:46 ON 21 OCT 2002
L1 127 S COCHLEATE
L2 230 S MULTILAMELLAR LIPID
L3 357 S L1 OR L2
L4 6610 S AAV OR ADENO ASSOCIATED
L5 1 S L3 AND L4
L6 5096 S REP!
L7 0 S L6 AND L3
L8 8791 S REP
L9 1 S L8 AND L3
L10 6979 S RECOMBINASE
L11 0 S L3 AND L10
L12 694865 S RECOMBIN?
L13 4 S L3 AND L12
L14 4 DUP REM L13 (0 DUPLICATES REMOVED)

=> s p47 phox
L15 910 P47 PHOX

=> s gene therapy
L16 65236 GENE THERAPY

=> s l15 and l16
L17 19 L15 AND L16

=> s l17 and review
L18 1 L17 AND REVIEW

=> d ti so

L18 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
TI Molecular pathology and **gene therapy** of chronic granulomatous disease
SO Saishin Igaku (1995), 50(Suppl. 601), 1785-94 CODEN: SAIGAK; ISSN: 0370-8241

=> dup rem l17
PROCESSING COMPLETED FOR L17
L19 16 DUP REM L17 (3 DUPLICATES REMOVED)

=> d ti so 1-16

L19 ANSWER 1 OF 16 MEDLINE
TI Statistical evaluation of chronic granulomatous disease in Japan and basic
studies for **gene therapy** for CGD patients.
SO RINSHO BYORI. JAPANESE JOURNAL OF CLINICAL PATHOLOGY, (1999 Jul) 47 (7)
658-64.
Journal code: 2984781R. ISSN: 0047-1860.

L19 ANSWER 2 OF 16 MEDLINE
TI **Gene therapy** for inherited diseases using
heamatopoietic stem cells--**gene therapy** for patients
with chronic granulomatous disease.
SO HUMAN CELL, (1999 Sep) 12 (3) 103-8. Ref: 18
Journal code: 8912329. ISSN: 0914-7470.

L19 ANSWER 3 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Drug-selected complete restoration of superoxide generation in
Epstein-Barr virus-transformed B cells from p47phox-deficient chronic
granulomatous disease patients by using a bicistronic retrovirus vector
encoding a human multi-drug resistance gene (MDR1) and the p47phox gene.
SO Human Genetics, (Oct., 1998) Vol. 103, No. 4, pp. 419-423.
ISSN: 0340-6717.

L19 ANSWER 4 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI The molecular basis of chronic granulomatous disease.
SO Springer Seminars in Immunopathology, (1998) Vol. 19, No. 4, pp. 417-434.
ISSN: 0172-6641.

L19 ANSWER 5 OF 16 MEDLINE DUPLICATE 1
TI Prolonged production of NADPH oxidase-corrected granulocytes after
gene therapy of chronic granulomatous disease.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
AMERICA, (1997 Oct 28) 94 (22) 12133-8.
Journal code: 7505876. ISSN: 0027-8424.

L19 ANSWER 6 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Enhanced host defense after gene transfer in the murine **p47-phox**-deficient model of chronic granulomatous disease.
SO Blood, (1997) Vol. 89, No. 7, pp. 2268-2275.
ISSN: 0006-4971.

L19 ANSWER 7 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Enhanced host defense after gene transfer in the murine **p47-phox**-deficient model of chronic granulomatous disease.
SO Blood, (1996) Vol. 88, No. 10 SUPPL. 1 PART 1-2, pp. 487A.
Meeting Info.: Thirty-eighth Annual Meeting of the American Society of
Hematology Orlando, Florida, USA December 6-10, 1996
ISSN: 0006-4971.

L19 ANSWER 8 OF 16 CAPLUS COPYRIGHT 2002 ACS
TI Molecular pathology and **gene therapy** of chronic granulomatous disease
SO Saishin Igaku (1995), 50(Suppl. 601), 1785-94
CODEN: SAIGAK; ISSN: 0370-8241

L19 ANSWER 9 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Functional reconstitution of the NADPH-oxidase by adeno-associated virus
gene transfer.
SO Blood, (1995) Vol. 86, No. 2, pp. 761-765.
ISSN: 0006-4971.

L19 ANSWER 10 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Generation of recombinant adeno-associated virus (rAAV) from an adenoviral
vector and functional reconstitution of the NADPH-oxidase.
SO Gene Therapy, (1995) Vol. 2, No. 7, pp. 481-485.
ISSN: 0969-7128.

L19 ANSWER 11 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Mouse model of chronic granulomatous disease (CGD): The **p47-phox** knock-out.
SO Journal of Investigative Medicine, (1995) Vol. 43, No. SUPPL. 2, pp. 287A.
Meeting Info.: Clinical Research Meeting San Diego, California, USA May
5-8, 1995

L19 ANSWER 12 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Peripheral blood progenitors as a target for genetic correction of p47-pho-deficient chronic granulomatous disease.
SO Proceedings of the National Academy of Sciences of the United States of
America, (1993) Vol. 90, No. 16, pp. 7446-7450.
ISSN: 0027-8424.

L19 ANSWER 13 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Restitution of superoxide generation in autosomal cytochrome-negative
chronic granulomatous disease (A22-0 CGD)-derived B lymphocyte cell lines
by transfection with p22-phox cDNA.
SO Journal of Experimental Medicine, (1993) Vol. 178, No. 6, pp. 2047-2053.
ISSN: 0022-1007.

L19 ANSWER 14 OF 16 MEDLINE DUPLICATE 2
TI Chronic granulomatous disease: towards **gene therapy**.
SO IMMUNODEFICIENCY, (1993) 4 (1-4) 327-33.
Journal code: 9418574. ISSN: 1067-795X.

L19 ANSWER 15 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Retrovirus mediated functional correction of superoxide production by EBV
transformed B cells from patients with **P47-PHOX**
deficient chronic granulomatous disease.
SO Journal of Allergy and Clinical Immunology, (1993) Vol. 91, No. 1 PART 2,
pp. 322.
Meeting Info.: Forty-ninth Annual Meeting of the American Academy of
Allergy and Immunology Chicago, Illinois, USA March 12-17, 1993
ISSN: 0091-6749.

L19 ANSWER 16 OF 16 MEDLINE DUPLICATE 3
TI Restoration of superoxide generation to a chronic granulomatous disease-derived B-cell line by retrovirus mediated gene transfer.
SO BLOOD, (1992 Sep 1) 80 (5) 1125-9.
Journal code: 7603509. ISSN: 0006-4971.

=> d ibib ab 1-3,5

L19 ANSWER 1 OF 16 MEDLINE
ACCESSION NUMBER: 1999370633 MEDLINE
DOCUMENT NUMBER: 99370633 PubMed ID: 10442045
TITLE: Statistical evaluation of chronic granulomatous disease

in

Japan and basic studies for **gene therapy** for CGD patients.
AUTHOR: Nunoi H; Ishibashi F
CORPORATE SOURCE: Department of Pediatrics, Kumamoto University School of Medicine.
SOURCE: RINSHO BYORI. JAPANESE JOURNAL OF CLINICAL PATHOLOGY, (1999 Jul) 47 (7) 658-64.
Journal code: 2984781R. ISSN: 0047-1860.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990921
Last Updated on STN: 19990921
Entered Medline: 19990903

AB Chronic granulomatous disease (CGD) is an inherited immune deficiency caused by mutations in any of the following four phox genes encoding subunits of the superoxide generating phagocyte NADPH oxidase. It consists of membranous cytochrome b558 composed of gp91-phox and p22-phox, and four cytosolic components, **p47-phox**, p67-phox, rac p21 and p40-phox, which translocate to the membrane upon activation. In our group study, more than 220 CGD patients has been enrolled. The incidence of CGD patients was estimated as 1 out of 250,000 births. The expected life span of the CGD patients is 25 to 30 years old by the Kaplan Meier analysis. Comparing with the ratio of CGD subtype in US and Europe, that with **p47-phox** deficiency is lower (less than 10% vs. 23%) and that of gp91-phox deficiency is higher (more than 75% vs. 60%). Prophylactic administration of ST antibiotics and IFN-gamma and bone marrow transplantation have been successfully employed in our therapeutic strategy. However, it is necessary to develop the **gene therapy** technology for CGD patients as more promising treatment. In the current study we constructed two retrovirus vectors; MFGS-gp91/293 SPA which contains only the therapeutic gp91-phox gene, a bicistronic retrovims pHa-MDR-IRES-gp91/PA317 which carries a multi drug resistant gene (MDR1) and the gp91-phox gene connected with an internal ribosome entry site (IRES). We demonstrate high efficiency transduction of gp91-phox to CGD EB virus established cell line with high levels of functional correction of the oxidase by MFGS-gp91 and by pHa-MDR-IRES-gp91, respectively. We also demonstrate sufficient transduction of gp91-phox to CD34+ haematopoietic stem cell from the patients with gp91-phox deficiency by MFGS-gp91/293 SPA. Our current studies suggest that the combination of the 293-SPA packaging system and the bicistronic retrovirus system inserted MDR1 gene make our CGD **gene therapy** more feasible for clinical application.

L19 ANSWER 2 OF 16 MEDLINE
ACCESSION NUMBER: 2000159339 MEDLINE
DOCUMENT NUMBER: 20159339 PubMed ID: 10695016
TITLE: **Gene therapy** for inherited diseases using heamatopoietic stem cells--**gene therapy** for patients with chronic granulomatous disease.
AUTHOR: Nunoi H; Ishibashi F
CORPORATE SOURCE: Department of Pediatrics, Kumamoto University Medical School, Japan.
SOURCE: HUMAN CELL, (1999 Sep) 12 (3) 103-8. Ref: 18
Journal code: 8912329. ISSN: 0914-7470.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000505
Last Updated on STN: 20000505
Entered Medline: 20000426

AB The possibility of **gene therapy** for inherited diseases with a single gene mutation in Figure 1 had been verified by the successful treatment with bone marrow transplantation. As the **gene therapy** method and theory has been progressing rapidly, it is expected that **gene therapy** will overcome the complications of bone marrow transplantation. Of these inherited diseases, chronic granulomatous disease (CGD) is the one of the most expected disease for **gene therapy**. CGD is an inherited immune deficiency caused by mutations in any of the following four phox genes encoding subunits of the superoxide generating phagocyte NADPH oxidase. It consists of membranous cytochrom b558 composed of gp91 phox and p22 phox, and four cytosolic components, **p47 phox**, p67 phox, rac p21 and p40 phox, which translocate to the membrane upon activation. In our group study, more than 220 CGD patients has been enrolled. The incidence of CGD patients was estimated as 1 out of 250,000 births. The expected life span of the CGD patients is 25 to 30 years old by the Kaplan Meier analysis. Comparing with the ratio of CGD subtype in US and Europe, that with p47phox deficiency is lower (less than 10%/o vs. 23%) and that of gp91 phox deficiency is higher (more than 75% vs. 60%). Prophylactic administration of ST antibiotics and IFN-gamma and bone marrow transplantation have been successfully employed in our therapeutic strategy. However, it is necessary to develop the **gene therapy** technology for CGD patients as more promising treatment. In the current study we constructed two retrovirus vectors; MFGS-gp91/293 SPA which contains only the therapeutic gp91 phox gene, a bicistronic retrovirus pHa-MDR-IRES-gp91/PA317 which carries a multi drug resistant gene (MDR1) and the gp91phox gene connected with an internal ribosome entry site (IRES). We demonstrate high efficiency transduction of gp 91 phox to CGD EB virus established cell line with high levels of functional correction of the oxidase by MFGS-gp91 and by pHa-MDR-IRES-gp91, respectively. We also demonstrate sufficient transduction of gp91 phox to CD34+ hematopoietic stem cell from the patients with gp91 phox deficiency by MFGS-gp91/293 SPA. Our current studies suggest that the combination of the 293-SPA packaging system and the bicistronic retrovirus system inserted MDR1 gene make our CGD **gene therapy** more feasible for clinical application.

L19 ANSWER 3 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL

[redacted]

ABSTRACTS INC.
ACCESSION NUMBER: 1999:48557 BIOSIS
DOCUMENT NUMBER: PREV199900048557
TITLE: Drug-selected complete restoration of superoxide generation
in Epstein-Barr virus-transformed B cells from p47phox-deficient chronic granulomatous disease patients by
using a bicistronic retrovirus vector encoding a human multi-drug resistance gene (MDR1) and the p47phox gene.
AUTHOR(S): Iwata, Mayumi; Nunoi, Hiroyuki; Matsuda, Ichiro; Kanegasaki, Shiro; Tsuruo, Takashi; Sugimoto, Yoshikazu (1)
CORPORATE SOURCE: (1) Cancer Chemotherapy Cent., Jpn. Found. Cancer Res.,
1-37-1 Kami-ikebukuro, Toshima-ku, Tokyo 170 Japan
SOURCE: Human Genetics, (Oct., 1998) Vol. 103, No. 4, pp. 419-423.
ISSN: 0340-6717.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Chronic granulomatous disease (CGD) is a group of disorders characterized
by the failure of phagocytes to produce superoxide. One-third of the cases
of CGD in the USA and Europe results from defects in the gene encoding
p47phox, a cytoplasmic component of NADPH oxidase for superoxide
generation. In this study, we constructed the bicistronic retrovirus vector Ha-MDR-IRES-p47, which carries cDNAs for a human multi-drug-resistance gene (MDR1) and p47phox. The amphotropic retroviral
producer cells were generated, and the supernatant of the producer cells
was used to transduce Epstein-Barr virus-transformed B (EBV-B) cells,
established from B cells of p47phox-deficient CGD patients, as an in vitro
model of **gene therapy** for p47phox-deficient CGD. The transduced cells expressed both P-glycoprotein and p47phox protein, and
the expression levels were increased after appropriate vincristine selection. The levels of superoxide production in the vincristine-selected
cells were increased to a level similar to normal EBV-B cells. This result
suggests that it is possible to achieve 100% correction of the CGD defect
in p47phox-deficient EBV-B cells by using the bicistronic vector. This
strategy could be employed not only in vitro, but also in vivo, in the **gene therapy** of a number of inherited diseases.

L19 ANSWER 5 OF 16 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1998004536 MEDLINE
DOCUMENT NUMBER: 98004536 PubMed ID: 9342375
TITLE: Prolonged production of NADPH oxidase-corrected granulocytes after **gene therapy** of
chronic granulomatous disease.
AUTHOR: Malech H L; Maples P B; Whiting-Theobald N; Linton G F;
Sekhsaria S; Vowells S J; Li F; Miller J A; DeCarlo E; Holland S M; Leitman S F; Carter C S; Butz R E; Read E J; Fleisher T A; Schneiderman R D; Van Epps D E; Spratt S K;
Maack C A; Rokovich J A; Cohen L K; Gallin J I
CORPORATE SOURCE: Laboratory of Host Defenses, National Institute of Allergy
and Infectious Diseases, National Institutes of Health, 10 Center Drive, MSC 1886, Bethesda, MD 20892, USA..
hmalech@nih.gov
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE
UNITED STATES OF AMERICA, (1997 Oct 28) 94 (22) 12133-8.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: (CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 19980109
Last Updated on STN: 19980109
Entered Medline: 19971204
AB Little is known about the potential for engraftment of autologous hematopoietic stem cells in human adults not subjected to myeloablative
conditioning regimens. Five adult patients with the **p47(phox)** deficiency form of chronic granulomatous disease received intravenous infusions of autologous CD34(+) peripheral blood stem cells
(PBSCs) that had been transduced ex vivo with a recombinant retrovirus
encoding normal **p47(phox)**. Although marrow conditioning was not given, functionally corrected granulocytes were detectable in peripheral blood of all five patients. Peak correction occurred 3-6 weeks after infusion and ranged from 0.004 to 0.05% of total
peripheral blood granulocytes. Corrected cells were detectable for as long
as 6 months after infusion in some individuals. Thus, prolonged engraftment of autologous PBSCs and continued expression of the transduced
gene can occur in adults without conditioning. This trial also piloted the
use of animal protein-free medium and a blood-bank-compatible closed
system of gas-permeable plastic containers for culture and transduction of
the PBSCs. These features enhance the safety of PBSCs directed **gene therapy**.

=> d his

(FILE 'HOME' ENTERED AT 11:48:35 ON 21 OCT 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 11:48:46 ON 21 OCT 2002
L1 127 S COCHLEATE
L2 230 S MULTILAMELLAR LIPID
L3 357 S L1 OR L2
L4 6610 S AAV OR ADENO ASSOCIATED
L5 1 S L3 AND L4
L6 5096 S REP!
L7 0 S L6 AND L3
L8 8791 S REP
L9 1 S L8 AND L3
L10 6979 S RECOMBINASE
L11 0 S L3 AND L10
L12 694865 S RECOMBIN?
L13 4 S L3 AND L12
L14 4 DUP REM L13 (0 DUPLICATES REMOVED)
L15 910 S P47 PHOX
L16 65236 S GENE THERAPY
L17 19 S L15 AND L16
L18 1 S L17 AND REVIEW
L19 16 DUP REM L17 (3 DUPLICATES REMOVED)

=> log hold
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 79.57 79.78

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)
SINCE FILE TOTAL

[redacted]

ENTRY SESSION
CA SUBSCRIBER PRICE -1.24 -1.24

SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 12:12:38 ON 21 OCT 2002

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1636DXS

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

********** Welcome to STN International **********

NEWS 1 Web Page URLs for STN Seminar Schedule - N. America
NEWS 2 Apr 08 "Ask CAS" for self-help around the clock
NEWS 3 Apr 09 BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 4 Apr 09 ZDB will be removed from STN
NEWS 5 Apr 19 US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 6 Apr 22 Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 7 Apr 22 BIOSIS Gene Names now available in TOXCENTER
NEWS 8 Apr 22 Federal Research in Progress (FEDRIP) now available
NEWS 9 Jun 03 New e-mail delivery for search results now available
NEWS 10 Jun 10 MEDLINE Reload
NEWS 11 Jun 10 PCTFULL has been reloaded
NEWS 12 Jul 02 FOREGE no longer contains STANDARDS file segment
NEWS 13 Jul 22 USAN to be reloaded July 28, 2002; saved answer sets no longer valid
NEWS 14 Jul 29 Enhanced polymer searching in REGISTRY
NEWS 15 Jul 30 NETFIRST to be removed from STN
NEWS 16 Aug 08 CANCERLIT reload
NEWS 17 Aug 08 PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 18 Aug 08 NTIS has been reloaded and enhanced
NEWS 19 Aug 19 Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 20 Aug 19 IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21 Aug 19 The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22 Aug 26 Sequence searching in REGISTRY enhanced
NEWS 23 Sep 03 JAPIO has been reloaded and enhanced
NEWS 24 Sep 16 Experimental properties added to the REGISTRY file
NEWS 25 Sep 16 Indexing added to some pre-1967 records in CA/CAPLUS
NEWS 26 Sep 16 CA Section Thesaurus available in CAPLUS and CA
NEWS 27 Oct 01 CASREACT Enriched with Reactions from 1907 to 1985
NEWS 28 Oct 21 EVENTLINE has been reloaded
NEWS 29 Oct 24 BEILSTEIN adds new search fields
NEWS 30 Oct 24 Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 31 Oct 25 MEDLINE SDI run of October 8, 2002 on STN

NEWS EXPRESS October 14 CURRENT WINDOWS VERSION IS V6.01,
CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP),
AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002
NEWS HOURS STN Operating Hours Plus Help Desk Availability
NEWS INTER General Internet Information
NEWS LOGIN Welcome Banner and News Items
NEWS PHONE Direct Dial and Telecommunication Network Access to STN
NEWS WWW CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

********** STN Columbus **********

FILE 'HOME' ENTERED AT 09:28:27 ON 28 OCT 2002

=> s transfect?
THIS COMMAND NOT AVAILABLE IN THE CURRENT FILE
Some commands only work in certain files. For example, the EXPAND command can only be used to look at the index in a file which has an index. Enter "HELP COMMANDS" at an arrow prompt (=>) for a list of
commands which can be used in this file.

=> file medline biosis caplus
COST IN U.S. DOLLARS SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST 0.21 0.21

FILE 'MEDLINE' ENTERED AT 09:28:46 ON 28 OCT 2002

FILE 'BIOSIS' ENTERED AT 09:28:46 ON 28 OCT 2002
COPYRIGHT (C) 2002 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'CAPLUS' ENTERED AT 09:28:46 ON 28 OCT 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

=> s transfect
L1 2605 TRANSFECT

=> s transfect?
L2 225394 TRANSFECT?

=> s rep 68 or rep68 or rep 78 or rep78
L3 466 REP 68 OR REP68 OR REP 78 OR REP78

=> s integrase
L4 6349 INTEGRASE

=> s l3 and l2
L5 70 L3 AND L2

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 42 DUP REM L5 (28 DUPLICATES REMOVED)

=> d ti so 1-42

L6 ANSWER 1 OF 42 MEDLINE
TI Studies of the mechanism of transactivation of the adeno-associated virus
p19 promoter by Rep protein.

SO JOURNAL OF VIROLOGY, (2002 Aug) 76 (16) 8225-35.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 2 OF 42 MEDLINE DUPLICATE 1
TI Herpes simplex virus type 1/adeno-associated virus hybrid vectors mediate
site-specific integration at the adeno-associated virus preintegration site, AAVS1, on human chromosome 19.
SO JOURNAL OF VIROLOGY, (2002 Jul) 76 (14) 7163-73.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 3 OF 42 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Hyper-phosphorylation of the adeno-associated virus **Rep78** protein inhibits terminal repeat binding and helicase activity.
SO Biochimica et Biophysica Acta, (19 July, 2002) Vol. 1576, No. 3, pp.
298-305. http://www.elsevier.com/locate/bba. print.
ISSN: 0006-3002.

L6 ANSWER 4 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI Site-specific transgene integration mediated by a hybrid adenovirus/adeno-associated virus vector using the Cre/loxP-expression-
switching system
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2

L6 ANSWER 5 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI Recombinant adenovirus expressing adeno-associated virus cap and rep
proteins supports production of high-titer recombinant adeno-associated
virus
SO Gene Therapy (2001), 8(9), 704-712
CODEN: GETHEC; ISSN: 0969-7128

L6 ANSWER 6 OF 42 MEDLINE
TI Site-specific integration of an adeno-associated virus vector plasmid mediated by regulated expression of rep based on Cre-loxP recombination.
SO JOURNAL OF VIROLOGY, (2000 Nov) 74 (22) 10631-8.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 7 OF 42 MEDLINE DUPLICATE 2
TI A chimeric protein containing the N terminus of the adeno-associated virus
Rep protein recognizes its target site in an in vivo assay.
SO JOURNAL OF VIROLOGY, (2000 Mar) 74 (5) 2372-82.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 8 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI Hormone-dependent forms of the adeno-associated virus Rep proteins and DNA
sequences and vectors coding for them and their use to regulate intracellular activity
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2

L6 ANSWER 9 OF 42 MEDLINE
TI Adeno-associated virus type 2 protein interactions: formation of pre-encapsidation complexes.
SO JOURNAL OF VIROLOGY, (1999 Nov) 73 (11) 8989-98.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 10 OF 42 MEDLINE
TI Enhancement of UV-induced cytotoxicity by the adeno-associated virus
replication proteins.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1999 Mar 19) 1444 (3) 371-83.
Journal code: 0217513. ISSN: 0006-3002.

L6 ANSWER 11 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI A conditional replication and expression system and its use for packaging
of adeno-associated virus vectors
SO PCT Int. Appl., 92 pp.
CODEN: PIXXD2

L6 ANSWER 12 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI Preparation of adeno-associated virus-derived vector for introducing genes
into animal cells using cre/loxP mechanism and its use in gene therapy
SO Jpn. Kokai Tokkyo Koho, 8 pp.
CODEN: JKXXAF

L6 ANSWER 13 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI A conditional replication and expression system
SO Eur. Pat. Appl., 33 pp.
CODEN: EPXXDW

L6 ANSWER 14 OF 42 MEDLINE DUPLICATE 3
TI Adeno-associated virus **Rep78** protein interacts with protein kinase A and its homolog PRKX and inhibits CREB-dependent transcriptional
activation.
SO JOURNAL OF VIROLOGY, (1998 Oct) 72 (10) 7916-25.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 15 OF 42 MEDLINE DUPLICATE 4
TI Rescue and autonomous replication of adeno-associated virus type 2 genomes
containing Rep-binding site mutations in the viral p5 promoter.
SO JOURNAL OF VIROLOGY, (1998 Jun) 72 (6) 4811-8.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 16 OF 42 MEDLINE DUPLICATE 5
TI Novel tools for production and purification of recombinant adenoassociated
virus vectors.
SO HUMAN GENE THERAPY, (1998 Dec 10) 9 (18) 2745-60.
Journal code: 9008950. ISSN: 1043-0342.

L6 ANSWER 17 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI Regulated gene expression in stably **transfected** mammalian cells using adeno-associated vectors containing inducible repressor and transactivator
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2

L6 ANSWER 18 OF 42 MEDLINE
TI Adeno-associated virus Rep proteins target DNA sequences to a unique locus
in the human genome.
SO JOURNAL OF VIROLOGY, (1997 Oct) 71 (10) 7951-9.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 19 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI High mobility group chromosomal protein 1 binds to the adeno-associated
virus replication protein (Rep) and promotes Rep-mediated site-specific
cleavage of DNA, ATPase activity and transcriptional repression
SO EMBO Journal (1997), 16(19), 5943-5954
CODEN: EMJODG; ISSN: 0261-4189

L6 ANSWER 20 OF 42 MEDLINE DUPLICATE 6
TI Adeno-associated virus **Rep78** protein and terminal repeats enhance integration of DNA sequences into the cellular genome.
SO JOURNAL OF VIROLOGY, (1997 Apr) 71 (4) 3299-306.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 21 OF 42 MEDLINE
TI The **Rep68** protein of adeno-associated virus type 2 increases RNA levels from the human cytomegalovirus major immediate early promoter.

[redacted]

SO VIROLOGY, (1997 Sep 15) 236 (1) 167-76.
Journal code: 0110674. ISSN: 0042-6822.

L6 ANSWER 22 OF 42 MEDLINE
TI The adeno-associated virus **Rep78** major regulatory/transformation suppressor protein binds cellular Sp1 in vitro and evidence of a biological effect.
SO CANCER RESEARCH, (1996 Nov 15) 56 (22) 5299-304.
Journal code: 2984705R. ISSN: 0008-5472.

L6 ANSWER 23 OF 42 MEDLINE
TI Identification of mutant adeno-associated virus Rep proteins which are
dominant-negative for DNA helicase activity.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1996 Mar 18) 220 (2)
294-9.
Journal code: 0372516. ISSN: 0006-291X.

L6 ANSWER 24 OF 42 MEDLINE
TI Role of the terminal repeat GAGC trimer, the major **Rep78** binding site, in adeno-associated virus DNA replication.
SO FEBS LETTERS, (1996 Nov 11) 397 (1) 97-100.
Journal code: 0155157. ISSN: 0014-5793.

L6 ANSWER 25 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI Stable cell lines capable of expressing the adeno-associated virus replication gene
SO PCT Int. Appl., 36 pp.
CODEN: PIXXD2

L6 ANSWER 26 OF 42 MEDLINE DUPLICATE 7
TI High-level expression of adeno-associated virus (AAV) **Rep78** or **Rep68** protein is sufficient for infectious-particle formation by a rep-negative AAV mutant.
SO JOURNAL OF VIROLOGY, (1995 Nov) 69 (11) 6880-5.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 27 OF 42 MEDLINE
TI Negative regulation of the adeno-associated virus (AAV) P5 promoter
involves both the P5 rep binding site and the consensus ATP-binding motif
of the AAV **Rep68** protein.
SO JOURNAL OF VIROLOGY, (1995 Nov) 69 (11) 6787-96.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 28 OF 42 MEDLINE DUPLICATE 8
TI Mutational analysis of adeno-associated virus Rep protein-mediated inhibition of heterologous and homologous promoters.
SO JOURNAL OF VIROLOGY, (1995 Sep) 69 (9) 5485-96.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 29 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI Sequence elements of the adeno-associated virus rep gene required for
suppression of herpes-simplex-virus-induced DNA amplification
SO Virology (1995), 206(1), 254-62
CODEN: VIRLAX; ISSN: 0042-6822

L6 ANSWER 30 OF 42 MEDLINE DUPLICATE 9
TI Cell lines inducibly expressing the adeno-associated virus (AAV) rep gene:
requirements for productive replication of rep-negative AAV mutants.
SO JOURNAL OF VIROLOGY, (1994 Nov) 68 (11) 7169-77.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 31 OF 42 MEDLINE
TI Analysis of adeno-associated virus (AAV) wild-type and mutant Rep proteins
for their abilities to negatively regulate AAV p5 and p19 mRNA levels.
SO JOURNAL OF VIROLOGY, (1994 May) 68 (5) 2947-57.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 32 OF 42 MEDLINE
TI Adeno-associated virus inhibits human papillomavirus type 16: a viral
interaction implicated in cervical cancer.
SO CANCER RESEARCH, (1994 Apr 15) 54 (8) 2278-81.
Journal code: 2984705R. ISSN: 0008-5472.

L6 ANSWER 33 OF 42 MEDLINE
TI Cloning, expression, and partial purification of **Rep78**: an adeno-associated virus replication protein.
SO VIROLOGY, (1994 May 1) 200 (2) 566-73.
Journal code: 0110674. ISSN: 0042-6822.

L6 ANSWER 34 OF 42 MEDLINE
TI Identification of a DNA-binding domain in the amino terminus of adeno-associated virus Rep proteins.
SO JOURNAL OF VIROLOGY, (1993 Feb) 67 (2) 997-1005.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 35 OF 42 MEDLINE DUPLICATE 10
TI In vitro resolution of adeno-associated virus DNA hairpin termini by wild-type Rep protein is inhibited by a dominant-negative mutant of rep.
SO JOURNAL OF VIROLOGY, (1992 Feb) 66 (2) 1236-40.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 36 OF 42 MEDLINE DUPLICATE 11
TI Inhibition of cellular transformation by the adeno-associated virus rep
gene.
SO VIROLOGY, (1991 Apr) 181 (2) 738-41.
Journal code: 0110674. ISSN: 0042-6822.

L6 ANSWER 37 OF 42 MEDLINE
TI Adeno-associated virus Rep protein inhibits human immunodeficiency virus
type 1 production in human cells.
SO JOURNAL OF VIROLOGY, (1991 Jan) 65 (1) 396-404.
Journal code: 0113724. ISSN: 0022-538X.

L6 ANSWER 38 OF 42 MEDLINE
TI Adeno-associated virus rep proteins produced in insect and mammalian
expression systems: wild-type and dominant-negative mutant proteins bind
to the viral replication origin.
SO VIROLOGY, (1991 Sep) 184 (1) 14-22.
Journal code: 0110674. ISSN: 0042-6822.

L6 ANSWER 39 OF 42 CAPLUS COPYRIGHT 2002 ACS
TI The adeno-associated virus rep gene suppresses herpes simplex virus-induced DNA amplification
SO Journal of Virology (1990), 64(6), 3012-18
CODEN: JOVIAM; ISSN: 0022-538X

L6 ANSWER 40 OF 42 MEDLINE DUPLICATE 12
TI The adeno-associated virus **Rep78** gene inhibits cellular transformation induced by bovine papillomavirus.
SO VIROLOGY, (1989 Sep) 172 (1) 253-61.
Journal code: 0110674. ISSN: 0042-6822.

L6 ANSWER 41 OF 42 MEDLINE DUPLICATE 13
TI Characterization of adeno-associated virus rep proteins in human cells by
antibodies raised against rep expressed in Escherichia coli.
SO VIROLOGY, (1987 Nov) 161 (1) 18-28.
Journal code: 0110674. ISSN: 0042-6822.

L6 ANSWER 42 OF 42 MEDLINE DUPLICATE 14
TI Identification of the trans-acting Rep proteins of adeno-associated virus
by antibodies to a synthetic oligopeptide.

SO JOURNAL OF VIROLOGY, (1986 Dec) 60 (3) 823-32.
Journal code: 0113724. ISSN: 0022-538X.

=> d his

(FILE 'HOME' ENTERED AT 09:28:27 ON 28 OCT 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 09:28:46 ON 28 OCT 2002
L1 2605 S TRANSFECT
L2 225394 S TRANSFECT?
L3 466 S REP 68 OR REP68 OR REP 78 OR REP78
L4 6349 S INTEGRASE
L5 70 S L3 AND L2
L6 42 DUP REM L5 (28 DUPLICATES REMOVED)

=> d ibib ab 20,18

L6 ANSWER 20 OF 42 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97214010 MEDLINE
DOCUMENT NUMBER: 97214010 PubMed ID: 9060699
TITLE: Adeno-associated virus **Rep78** protein and terminal repeats enhance integration of DNA sequences into the cellular genome.
AUTHOR: Balague C; Kalla M; Zhang W W
CORPORATE SOURCE: Molecular Biology Department, Baxter Healthcare Corporation, Round Lake, Illinois 60073, USA.. balaguc@baxter.com
SOURCE: JOURNAL OF VIROLOGY, (1997 Apr) 71 (4) 3299-306.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970424
Last Updated on STN: 19970424
Entered Medline: 19970411
AB Two adeno-associated virus (AAV) elements are necessary for the integration of the AAV genome: **Rep78**/68 proteins and inverted terminal repeats (ITRs). To study the contribution of the Rep proteins and the ITRs in the process of integration, we have compared the integration efficiencies of three different plasmids containing a green fluorescent protein (GFP) expression cassette. In one plasmid, no viral sequences were present; a second plasmid contained AAV ITRs flanking the reporter gene (integration cassette), and a third plasmid consisted of an integration cassette plus a **Rep78** expression cassette. One day after **transfection** of 293 cells, fluorescent cells were sorted by flow cytometry and plated at 1 cell per well. Two weeks after sorting, colonies were monitored for stable expression of GFP. **Transfection** with the GFP plasmid containing no viral sequences resulted in no stable fluorescent colonies. **Transfection** with the plasmid containing the integration cassette alone (GFP flanked by ITRs) produced stable fluorescent colonies at a frequency of 5.3% +/- 1.0% whereas **transfection** with the plasmid containing both the integration cassette and **Rep78** expression cassette produced stable fluorescent colonies at a frequency of 47% +/- 7.5%. Southern blot analysis indicated that in the presence of **Rep78**, integration is targeted to the AAVS1 site in more than 50% of the clones analyzed. Some clones also showed tandem arrays of the integrated GFP cassette. Both head-to-head and head-to-tail orientations were detected. These findings indicate that the presence of AAV ITRs and the **Rep78** protein enhance the integration of DNA sequences into the cellular genome and that the integration cassette is targeted to AAVS1 in the presence of **Rep78**.

L6 ANSWER 18 OF 42 MEDLINE
ACCESSION NUMBER: 97456572 MEDLINE
DOCUMENT NUMBER: 97456572 PubMed ID: 9311886
TITLE: Adeno-associated virus Rep proteins target DNA sequences to a unique locus in the human genome.
AUTHOR: Surosky R T; Urabe M; Godwin S G; McQuiston S A; Kurtzman G J; Ozawa K; Natsoulis G
CORPORATE SOURCE: Avigen, Inc., Alameda, California 94502, USA.. surosky@avigen.com
CONTRACT NUMBER: U01-AI35382-01 (NIAID)
SOURCE: JOURNAL OF VIROLOGY, (1997 Oct) 71 (10) 7951-9.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971020
AB We have developed a system for site-specific DNA integration in human cells, mediated by the adeno-associated virus (AAV) Rep proteins. In its normal lysogenic cycle, AAV integrates at a site on human chromosome 19 termed AAVS1. We describe a rapid PCR assay for the detection of integration events at AAVS1 in whole populations of cells. Using this assay, we determined that the AAV Rep proteins, delivered in cis or trans, are required for integration at AAVS1. Only the large forms of the Rep protein, **Rep78** and **Rep68**, promoted site-specific integration. The AAV inverted terminal repeats, present in cis, were not essential for integration at AAVS1, but in cells containing Rep, they increased the efficiency of integration. In the presence of the Rep proteins, the integration of a plasmid containing AAV inverted terminal repeats occurred at high frequency, such that clones containing the plasmid could be isolated without selection. In two of the five clones analyzed by fluorescence in situ hybridization, the plasmid DNA was integrated at AAVS1. In most of the clones, at least one copy of the entire plasmid was integrated in a tandem array. Detailed analysis of the integrated plasmid structure in one clone suggested a complex mechanism producing rearrangements of the flanking genomic DNA, similar to those observed with wild-type AAV.

=> d his

(FILE 'HOME' ENTERED AT 09:28:27 ON 28 OCT 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 09:28:46 ON 28 OCT 2002
L1 2605 S TRANSFECT
L2 225394 S TRANSFECT?
L3 466 S REP 68 OR REP68 OR REP 78 OR REP78
L4 6349 S INTEGRASE
L5 70 S L3 AND L2
L6 42 DUP REM L5 (28 DUPLICATES REMOVED)

=> s cotransfect? or co-transfect?

L7 22034 COTRANSFECT? OR CO-TRANSFECT?

=> s l4 and l7
L8 47 L4 AND L7

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9 23 DUP REM L8 (24 DUPLICATES REMOVED)

=> s l9 not py>1998
L10 5 L9 NOT PY>1998

=> d ti so 1-5

L10 ANSWER 1 OF 5 MEDLINE
TI Extensive regions of pol are required for efficient human immunodeficiency
virus polyprotein processing and particle maturation.
SO VIROLOGY, (1996 May 1) 219 (1) 29-36.
Journal code: 0110674. ISSN: 0042-6822.

L10 ANSWER 2 OF 5 MEDLINE
TI The nonmyristylated Pr160gag-pol polyprotein of human immunodeficiency
virus type 1 interacts with Pr55gag and is incorporated into viruslike particles.
SO JOURNAL OF VIROLOGY, (1992 Nov) 66 (11) 6304-13.
Journal code: 0113724. ISSN: 0022-538X.

L10 ANSWER 3 OF 5 MEDLINE
TI A rat brain mRNA encoding a transcriptional activator homologous to the
DNA binding domain of retroviral **integrases**.
SO NUCLEIC ACIDS RESEARCH, (1991 Oct 11) 19 (19) 5269-74.
Journal code: 0411011. ISSN: 0305-1048.

L10 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2002 ACS
TI A system to analyze and identify inhibitors of HIV-1 gene regulation using
a defective integrated provirus
SO Methods in Enzymology (1996), 275(Viral Polymerases and Related Proteins),
348-361
CODEN: MENZAU; ISSN: 0076-6879

L10 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2002 ACS
TI Autonomous transposition of the tobacco retrotransposon Ttol in rice
SO Plant Cell (1996), 8(4), 725-34
CODEN: PLCEEW; ISSN: 1040-4651

=> d his

(FILE 'HOME' ENTERED AT 09:28:27 ON 28 OCT 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 09:28:46 ON 28 OCT 2002
L1 2605 S TRANSFECT
L2 225394 S TRANSFECT?
L3 466 S REP 68 OR REP68 OR REP 78 OR REP78
L4 6349 S INTEGRASE
L5 70 S L3 AND L2
L6 42 DUP REM L5 (28 DUPLICATES REMOVED)
L7 22034 S COTRANSFECT? OR CO-TRANSFECT?
L8 47 S L4 AND L7
L9 23 DUP REM L8 (24 DUPLICATES REMOVED)
L10 5 S L9 NOT PY>1998

=> log hold
COST IN U.S. DOLLARS

	ENTRY	SINCE FILE SESSION	TOTAL
FULL ESTIMATED COST		45.60	45.81

SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 09:39:15 ON 28 OCT 2002

Teaches conversion
102 Cell Type
Can use for DP.

US-PAT-NO: 5643574

DOCUMENT-IDENTIFIER: US 5643574 A

TITLE: Protein- or peptide-cochleate vaccines and methods of immunizing using the same

DATE-ISSUED: July 1, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gould-Fogerite; Susan	Annandale	NJ	N/A	N/A
Mannino; Raphael James	Annandale	NJ	N/A	N/A

ASSIGNEE INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Albany Medical College	Albany	NY	N/A	N/A	02
University of Medicine & Dentistry of New Jersey	Newark	NJ	N/A	N/A	02

APPL-NO: 08/ 130986

DATE FILED: October 4, 1993

INT-CL: [06] A61K039/00;A61K051/00 ;A61K038/16 ;B01J013/02

US-CL-ISSUED: 424/184.1;424/121 ;264/46 ;514/8

US-CL-CURRENT: 424/184.1; 264/4.6 ; 424/1.21 ; 514/8

FIELD-OF-SEARCH: 424/88; 424/89 ; 424/92 ; 424/184.1 ; 424/1.21 ; 264/4.6 ; 514/8

REF-CITED:

U.S. PATENT DOCUMENTS

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
4078052	March 1978	Papahadjopoulos	424/36
	N/A	N/A	N/A
4663161	May 1987	Mannino et al.	N/A
	N/A	N/A	N/A
4871488	October 1989	Mannino et al.	N/A
	N/A	N/A	N/A

OTHER PUBLICATIONS

Miller et al, Vaccination of Rhesus Monkeys with Synthetic Peptide in a Fusogenic Proteoliposome Elicits Simian Immunodeficiency Virus-Specific CD8+ Cytotoxic T Lymphocytes, J. Exp. Med., vol. 176, pp. 1739-1744, 1992.

R. J. Mannino and S. Gould-Fogerite, Liposome Mediated Gene Transfer, BioTechniques, vol. 6, No. 7, pp. 682-690, 1988.

Gould-Fogerite et al, Chimerasome-mediated gene transfer in vitro and in vivo, Gene, vol. 84, pp. 429-438, 1989.

S. Gould-Fogerite and R.J. Mannino, Rotary Dialysis: Its Application to the Preparation of Large-Liposomes and Large Proteoliposomes (Protein-Lipid Vesicles) with High Encapsulation Efficiency and Efficient Reconstitution of Membrane Proteins, Analytical Biochemistry, vol. 148, pp. 15-25, 1985.

Gould-Fogerite et al, The Reconstitution of Biologically Active Glycoproteins into Large Liposomes: Use as a Delivery Vehicle to Animal Cells, Advances in Membrane Biochemistry and Bioenergetics, pp. 569-586, 1988.

S. Gould-Fogerite and R.J. Mannino, Liposome Preparation and Related Techniques, Liposome Technology 2nd Edition, vol. I, pp. 67-80.

[redacted]

S. Gould-Fogerite and R.J. Mannino, Entrapment of Drugs and Other
Materials, Liposome Technology 2nd Edition, vol. II, pp. 167-184.

S. Gould-Fogerite and R.J. Mannino, Interactions of Liposomes with the
Biological Milieu, Liposome Technology 2nd Edition, vol. III, pp. 261-276.

G. Goodman-Snitkoff et al, Defining Minimal Requirements for Antibody
Production to Peptide Antigens, Vaccine, vol. 8, pp. 257-262, 1990.

G. Goodman-Snitkoff et al, Role of Intrastructural/Intermolecular Help in
Immunization with Peptide-Phospholipid Complexes, The Journal of Immunology,
vol. 147, No. 2, pp. 410-415, 1991.

D. Papahadjopoulos et al, Biochem. Biophys. Acta, vol. 394, p. 483, 1975.

Liposome Technology, Second Edition, vol. 1, G. Gregoriadis, Ph.D.,
"Liposome Preparation and Related Techniques", published 1993 by CRC Press,
Inc., (Boca Raton), pp. 67-80.

J. Exp. Med., vol. 176, issued Dec. 1992, M.D. Miller et al, "Vaccination
of Rhesus Monkeys with Synthetic Peptide in a Fusogenic Proteoliposome Elicits
Simian Immunodeficiency Virus-specific CD8+ Cytotoxic T Lymphocytes", pp.
1739-1744.

Nature, vol. 342, issued 30 Nov. 1989, K. Deres et al, "In vivo Priming of
Virus-specific Cytotoxic T lymphocytes with Synthetic Lipopeptide Vaccine", pp.
561-564.

Biochimica et Biophysica Acta, vol. 394, issued 1975, D. Papahadjopoulos et
al, "Cocheleate Lipid Cylinders: Formation By Fusion of Unilamellar Lipid

[redacted]

Vesciles", pp. 483-491.

C. H. Kim et al, "Advances in Membrane Biochemistry and Bioenergetics" published 1988 by Plenum Publishing Corporation, pp. 569-586.

ART-UNIT: 182

PRIMARY-EXAMINER: Housel; James C.

ASSISTANT-EXAMINER: Minnifield; N. M.

ABSTRACT:

A method is described of immunizing a host by administering a biologically effective amount of a protein- or peptide-cochleate comprising at least a protein or peptide to which an immune response is elicited, a negatively charged lipid, and a divalent cation.

26 Claims, 25 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 25

GOVT-INTEREST:
Portions of the subject matter disclosed herein were supported in part by monies or grants from the United States Government.

BRIEF SUMMARY:

(1) FIELD OF THE INVENTION

(2) The present invention relates to protein- or peptide-cochleate vaccines and methods of immunizing using protein- or peptide-cochleate structures. These unique vaccines are composed of insoluble antigen-lipid-divalent cation structures which can be administered orally as well as by conventional routes and which generate mucosal as well as circulating immune responses. Protective

immunity against live pathogen challenge on a mucosal surface is demonstrated.

(3) BACKGROUND OF THE INVENTION

(4) Plain lipid cochleates (FIG. 1) have been described previously. Protein- or peptide-cochleates have been described heretofore and patented by the present inventors, as intermediate structures which can be converted to protein-lipid vesicles (proteoliposomes) (FIG. 2) by the addition of calcium chelating agents (see U.S. Pat. No. 4,663,161 and U.S. Pat. No. 4,871,488, the disclosures of which are expressly incorporated herein by reference). The structure of a protein- or peptide-cochleate is thought to be similar, perhaps with protrusions or bulges around the protein or peptide moieties. Indeed, a freeze-fracture electron micrograph of cochleates containing Sendai glycoproteins made by the DC method shows the rolled up lipid bilayer structures with a "bumpy" surface (FIG. 3). Plain phospholipid cochleates are smooth in this type of preparation. These proteoliposomes resulting from protein- or peptide-cochleates have been shown to be effective immunogens when administered to animals by intraperitoneal and intramuscular routes of immunization (G. Goodman-Snitkoff, et al., J. Immunol., Vol. 147, p.410 (1991); M. D. Miller, et al., J. Exp. Med., Vol. 176, p. 1739 (1992)). Further, when the glycoproteins of Sendai or influenza viruses are reconstituted by this method, these proteoliposomes are effective delivery vehicles for proteins and DNA to animals and to cells in culture (R. J. Mannino and S. Gould-Fogerite, Biotechniques, Vol. 6, No. 1, pp. 682-690 (1988); S. Gould-Fogerite et al., Gene, Vol. 84, p. 429 (1989); M. D. Miller, et al., J. Exp. Med., Vol. 176, p. 1739 (1992)). Nonetheless, it would be advantageous to

provide additional configurations for synthetic vaccines. It would also be advantageous to provide synthetic vaccines in a form that is stable at room temperature and that is suitable for oral administration. As a result of investigations in this area, the present invention was made.

(5) SUMMARY OF THE INVENTION

(6) Accordingly, it is an object of this invention to provide vaccines and a method of immunizing, wherein the vaccine is composed of an insoluble antigen-lipid-divalent cation structure which, following administration, including oral, i.e., peroral, administration, can induce mucosal and circulating, humoral and cell mediated immune responses.

(7) These and other objects have been obtained by providing a vaccine comprising an immunologically effective amount of a protein- or peptide-cochleate, wherein said protein- or peptide-cochleate comprises the following components:

(8) a) a protein or peptide component to which an immune response can be elicited,

(9) b) a negatively charged lipid component, and

(10) c) a divalent cation component.

(11) The present invention also provides a method of immunizing comprising administering to a host a biologically effective amount of the above-described protein- or peptide-cochleate.

(12) In a preferred embodiment, the vaccine is administered orally.

(13) The advantages of immunizing with cochleates are numerous. The

cochleates have a non-aqueous structure and therefore they:

(14) (a) are more stable because of less oxidation of lipids;

(15) (b) can be stored lyophilized which provides the potential to be stored for long periods of time at room temperatures, which would be advantageous for worldwide shipping and storage prior to administration;

(16) (c) maintain their structure even after lyophilization, whereas liposome structures are destroyed by lyophilization;

(17) (d) exhibit efficient incorporation of antigens with hydrophobic moieties into the lipid bilayer of the cochleate structure;

(18) (e) have the potential for slow release of antigen in vivo as cochleates slowly unwind or otherwise dissociate;

(19) (f) have a lipid bilayer matrix which serves as a carrier and is composed of simple lipids which are found in animal and plant cell membranes, so that the lipids are non-toxic, non-immunogenic and non-inflammatory;

(20) (g) contain high concentration of calcium, an essential mineral;

(21) (h) are safer than live vaccines, since the cochleates are non-living subunit formulations, and as a result the cochleates have none of the risks associated with use of live vaccines, such as life threatening infections in immunocompromised individuals or reversion to wild type infectivity which poses a danger to even healthy people;

(22) (i) are produced easily and safely; and

(23) (j) can be produced as defined formulations composed of predetermined amounts and ratios of antigens, including proteins,

peptides, carbohydrates, and nucleic acids.

(24) The advantages of oral vaccination are also numerous. An oral route has been chosen by the WHO Children's Vaccine Initiative because of ease of administration and opportunity to prime the mucosal immune system. Oral vaccines are less expensive and much safer to administer than parenterally (intramuscular or subcutaneous) administered vaccines. The use of needles adds to the cost, and also, unfortunately, in the field, needles are often reused. This can lead to spread of disease between vaccinated individuals and could be potentially disastrous in areas where there is a high incidence of infection with human immunodeficiency virus (HIV) which causes AIDS. Further, oral, nasal, ocular and vaginal mucous membranes are the primary routes of entry for a large number and wide variety of human disease-causing agents. Intramuscular or subcutaneous administration of vaccines often does not lead to significant protection against these infectious agents. In contrast, the oral route of delivery can stimulate strong protective responses on mucous membranes and in the circulation.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic representation of a plain lipid cochleate.

FIG. 2 shows the structure of protein-lipid vesicles with integrated membrane proteins.

FIG. 3 is a freeze-fracture electron micrograph of a protein- or peptide-cochleate. The inset bar is 0.1 micrometers.

FIG. 4 summarizes the various alternative procedures for the preparation of

protein- or peptide-cochleates.

FIGS. 5(A) and 5(B) show serum antibody titers in mice following oral administration of influenza protein-cochleates.

FIG. 6 is a graph showing serum antibody titers following a single oral dose of influenza protein-cochleates.

FIG. 7 is a graph showing the results of oral administration of protein- or peptide-cochleares when challenged with live virus. In the figure, "ND" means "not determined".

FIG. 8 is a graphic representation of serum antibody titers in mice following oral administration of Sendai-cochleates.

FIG. 9 is a graph showing antibody-isotypes following oral administration of Sendai protein cochleates.

FIG. 10 is a graph showing antigen-specific IgA following oral administration of Sendai protein cochleates.

FIG. 11 is a graph showing the production of antigen-specific local or secretory IgA after three immunizations with protein-cochleates.

FIGS. 12(A) and (B), 13(A) and (B), 14(A) and (B), 15(A) and (B), and 16(A) and (B) show spleenocite proliferation following immunization with influenza-cochleates. Part (A) of each figure shows the response to media as a control. Part (B) of each figure shows the proliferative response to ultraviolet light-inactivated influenza virus over several days in culture.

FIG. 17 is a graph depicting the induction of antigen specific cytotoxic spleenocites following oral administration of Sendai-cochleates.

FIG. 18 shows the antibody responses following oral administration of cochleates containing Sendai glycoproteins, a peptide linked to phosphatidylethanolamine or both Sendai and PE-linked peptide.

FIG. 19 is a graph showing Peyer's Patch proliferation after oral administration of Sendai-cochleates.

(1) DETAILED DESCRIPTION OF THE INVENTION

(2) The present inventors have now found surprisingly and have demonstrated that protein- or peptide-cochleate structures can themselves be used as vaccines, including oral vaccines. These cochleates apparently survive the harsh acid environment of the stomach, protecting the delicate proteins within them, probably by virtue of their unique multilayered precipitate structure. It is likely that they are then taken up by microfold cells (M cells) in the small intestine, where they are presented to T and B cells. Appropriate stimulation of these cells by foreign proteins can lead to blood borne (circulatory) and mucous membrane borne (mucosal) immune responses. These can be humoral (antibody) and cell mediated (helper or cytotoxic "killer" cell) responses.

(3) The present inventors have demonstrated that oral administration by drinking cochleates containing the glycoproteins and viral lipids from the surface of influenza or Sendai viruses plus phosphatidyl serine and cholesterol, stimulate both mucosal and circulating antibody responses. In addition, strong helper cell (proliferative) and killer (cytotoxic) cell responses are also generated. Perhaps most impressively, oral administration

of the influenza cochleates has been shown to protect against intranasal challenge with live virus.

(4) These results are unexpected for a number of reasons.

(5) It was not known and was not expected that the cochleate structures would survive the stomach and protect the proteins associated with them from its acid environment and degradative enzymes. It is known that without the presence of at least 3 mM calcium, the cochleates begin to unwind and form liposomes. It was possible, in fact likely, that the cochleates would not remain intact during the transit from the mouth, down the esophagus, and through the stomach. If they did come apart, they would be digested as food.

(6) Despite the attractiveness of the oral route for vaccine administration because of its ease and the possibility of priming the mucosal immune system, very little success has been achieved in this area. Positive results have mainly been limited to viruses and bacteria which have evolved to infect using the oral route of entry to eventually replicate in the gut, (e.g., polio virus). Enveloped viruses such as influenza and Sendai (and liposomes made from them) do not have the appropriate physical characteristics to efficiently survive the stomach and small intestinal degradation environments. Additionally, it has been difficult to achieve significant circulatory immune responses using nonliving vaccines administered only by the oral route. Some success has been achieved using multiple intramuscular priming boosts and then following with oral boosting. To the inventors' knowledge, the present invention is the only system where oral administration of a subunit vaccine which is not or does not contain parts of an organism which infects the

[redacted]

gastrointestinal tract has led to significant circulating and mucosal antibody responses, and cell mediated immunity. The fact that the mucosal (and circulating) responses were significant enough to protect mice from viral replication in the trachea and lungs following intranasal challenge, makes these results all the more novel and significant.

(7) Also, having survived the stomach, that these structures would interact in an effective way with the mucosal and circulating immune systems was unknown and unexpected. Everyone ingests large quantities of proteins, fats and sugars on a daily basis which simply get digested and used as fuel, without stimulating any kind of mucosal or circulating immune responses. Yet, the body needs to be able to respond to infectious organisms which enter and infect by this route. The parameters which regulate the outcome of introduction of proteins via this route, i.e., immune response, lack of response, or tolerance, are not currently understood. Given the difficulty in using the oral route to get good immune responses to non-live vaccines, and the lack of understanding of the regulatory mechanisms involved, the ability to use cochleate structures to induce strong circulating and mucosal immune responses could not be predicted.

(8) As used herein, the term "immune response" means either antibody, cellular, proliferative, or cytotoxic activities, or secretion of cytokines.

(9) The protein- or peptide-cochleates used in the vaccine and method according to the present invention can be prepared by known methods such as those described in U.S. Pat. No. 4,663,161, filed Apr. 22, 1985, U.S. Pat. No. 4,871,488, filed Apr. 13, 1987, S. Gould-Fogerite et

al., Analytical Biochemistry, Vol. 148, pages 15-25 (1985); S. Gould-Fogerite et al., Advances in Membrane Biochemistry and Bioenergetics, edited by Kim, C. H., Tedeschi, T., Diwan, J. J., and Salerno, J. C., Plenum Press, New York, pages 569-586 (1988); S. Gould-Fogerite et al., Gene, Vol. 84, pages 429-438 (1989); Liposome Technology, 2nd Edition, Vol. I, Liposome Preparation and Related Techniques, Vol. II, Entrapment of Drugs and Other Materials, and Vol. III, Interactions of Liposomes with the Biological Milieu, all edited by Gregory Gregoriadis (CRC Press, Boca Raton, Ann Arbor, London, Tokyo), Chapter 4, pp 69-80, Chapter 10, pp 167-184, and Chapter 17, pp. 261-276 (1993); and R. J. Mannino and S. Gould-Fogerite, Liposome Mediated Gene Transfer, Biotechniques, Vol. 6, No. 1 (1988), pp. 682-690. In the initial step of these methods, a desired immunogen which can be a peptide or protein, a carbohydrate, or DNA, is prepared.

(10) The immunogen is extracted out from the source particle, cell, tissue, or organism by known methods. Preferably the immunogen is a peptide or protein. Preferably the peptide or protein is a glycoprotein or membrane protein, and more preferably a membrane glycoprotein. Biological activity of proteins need not be maintained. However, in some instances (e.g., where a protein has membrane fusion or ligand binding activity or a complex conformation which is recognized by the immune system), it is desirable to maintain the biological activity of a protein. In these instances, an extraction buffer containing a detergent which does not destroy the biological activity of the membrane protein is used. Suitable detergents include ionic detergents such as cholate salts, deoxycholate salts and

[redacted]

the like or nonionic detergents such as those containing polyoxyethylene or sugar head groups or heterogeneous polyoxyethylene detergents such as TWEEN or BRIG or TRITON. Preferred detergents are nonionic detergents containing sugar head groups such as the alkyl glucosides. A particularly preferred nonionic detergent for this purpose is .beta.-D-octyl-glucopyranoside.

(11) Utilization of this method allows efficient association with the cochleates and, eventually, reconstitution of the membrane proteins into the liposomes with retention of biological activities. This step avoids previously utilized organic solvents, sonication, or extremes of pH, temperature, or pressure, all of which may have an adverse effect upon efficient reconstitution in a biologically active form of the desired membrane proteins.

(12) The buffer component utilized in conjunction with the aforesaid detergents can be any conventional buffer employed for membrane protein extractions. A suitable extraction buffer for the present purposes can be prepared utilizing a 2M NaCl, 0.02M sodium phosphate buffer (pH 7.4). The concentration of the detergent component in the buffer is not narrowly critical and can be in the range of from 0.1 to 20% (w/v) preferably from 1 to 5%, most preferably about 2%. The extraction efficiency can be enhanced by utilizing techniques well known in the art, such as by vortexing and sonicating.

(13) The extracted membrane proteins are removed from nonsoluble debris by procedures well known in the art, such as for example by centrifugation or chromatography. The resulting supernatant containing the extracted membrane protein may then be applied directly in the cochleate

formation procedure.

(14) Membrane proteins which can be employed in the practice of the present invention include viral proteins such as for example viral envelope protein, animal cell membrane protein, plant cell membrane protein, bacterial membrane protein, parasite membrane protein, viral membrane protein and the like. These respective proteins can be separated from other components by procedures well known in the art prior to introduction into the present methodology or they can be resolved during the course of the procedure as will be described below.

(15) Suitable sources of viral proteins which can be employed in conjunction with the method of the invention include Sendai, influenza, herpes simplex or genitalis, HTLV I, II or III, retroviruses, pox virus, respiratory syncytial virus, toga virus, and the like. The present invention can also be employed in conjunction with membrane proteins derived from bacterial or parasitic organisms such as for example organisms causing malaria, chlamydia, N. gonorrhea, salmonella, liver flukes and the like.

(16) Peptides or proteins having at least enough hydrophobic character to allow for association with a lipid bilayer are preferred. Additionally, the peptides or proteins can be covalently cross-linked to a lipid as described in Liposome Technology, 2nd Edition, Vol. II, Entrapment of Drugs and Other Materials, edited by Gregory Gregoriadis, (CRC Press, Boca Raton, Ann Arbor, London, Tokyo), Chapter 10, pages 167-184 (1993).

(17) Examples of suitable peptides and proteins can be found in the following references:

(18) (1) G. Goodman-Snitkoff et al., Defining Minimal

[redacted]

Requirements for
Antibody Production to Peptide Antigens, Vaccine, Vol. 8, page 257 (1990);

(19) (2) G. Goodman-Snitkoff et al., Role of Intrastructural/Intermolecular
Help in Immunization with Peptide-Phospholipid complexes, J. Immunol., Vol.
147, page 410 (1991);

(20) (3) R. J. Mannino et al., Liposomes as Adjuvants for Peptides:
Preparation and Use of Immunogenic Peptide-phospholipid Complexes, in Liposome
Technology, 2nd Edition, Vol. II, Entrapment of Drugs and other Materials,
edited by Gregory Gregoriadis, (CRC Press, Boca Raton, Ann Arbor, London,
Tokyo), Ch. 10, pp. 167-184 (1993).

(21) The aforesaid peptides or proteins, or mixtures thereof to provide
multiple epitopes, are then mixed with phospholipid to form protein- or
peptide-cochleates. Carbohydrate or DNA immunogen can also be added.

(22) In order to form cochleate precipitates, a majority of the lipid present
must be negatively charged. One type of lipid can be used or a mixture of
lipids can be used. Phosphatidylserine or
phosphatidyl-glycerol have generally
been used. Phosphatidyl-inositol also forms a precipitate which converts to
liposomes upon contact with EDTA. A substantial proportion of the lipid can,
however, be neutral or positively charged. The present inventors have included
up to 40 mol % cholesterol based on total lipid present and routinely make
protein-lipid cochleates which contain 10 mol % cholesterol and 20% viral
membrane lipids. Phosphatidylethanolamine, plain or cross-linked to peptides
or proteins, can also be incorporated into cochleates.

(23) While negatively charged lipid can be used, a

negatively charged phospholipid is preferred, and of these phosphatidylserine, phosphatidylinositol, phosphatidic acid, and phosphatidylglycerol are most preferred.

(24) One skilled in the art can readily determine how much lipid must be negatively charged by preparing a mixture with known concentrations of negative and non-negative lipids and by any of the procedures described below, determining whether precipitates form.

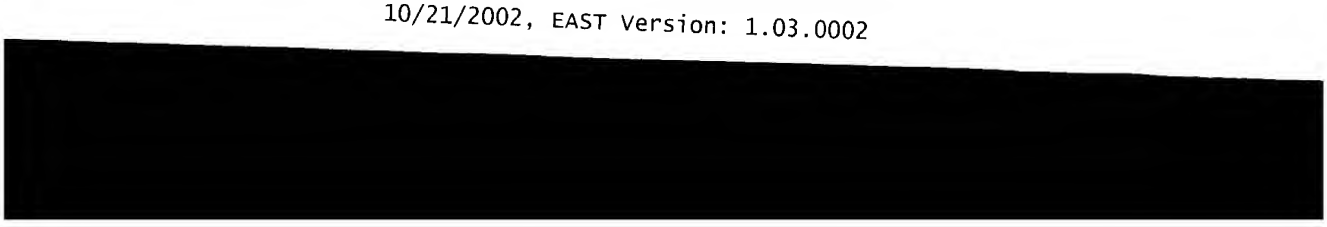
(25) There are several known procedures for making the protein- or peptide-cochleates of the present invention and these are schematized in FIG. 4. One such method is the so-called standard cochleate obtained by use of the calcium-EDTA-chelation technique described by D. Papahadjopoulos et al. [Biochem. Biophys. Acta, Vol. 394, page 483 (1975)] for making plain phospholipid cochleates. In an embodiment of the present invention, a modification of such procedure is employed. In the modified procedure a negatively charged lipid such as phosphatidylserine, phosphatidylinositol, phosphatidic acid or phosphatidylglycerol in the absence or presence of cholesterol (up to 3:1, preferably 9:1 w/w) are utilized to produce a suspension of multilamellar protein lipid vesicles containing and surrounded by antigen (protein, carbohydrate, and/or DNA) which are converted to small unilamellar protein lipid vesicles by sonication under nitrogen. These vesicles are dialyzed at room temperature against buffered divalent cation, e.g., calcium chloride, resulting in the formation of an insoluble precipitate referred to as a cochleate cylinder. After centrifugation, the resulting pellet can be taken up in buffer to yield the cochleate solution utilized in

the vaccine of the present invention.

(26) In an alternative and preferred embodiment, an amount of negatively charged lipid, e.g., phosphatidylserine, and cholesterol in the same proportions as above and equal to from about 1 to 10 times the weight, preferably equal to four times the weight of the viral or other additional lipids are utilized to prepare the cochleates. Supernatant from the nonionic detergent extraction of membrane proteins or other proteins or peptides is then added, and the solution is vortexed for five minutes. Either carbohydrates or DNA can be used in place of or in combination with peptides or proteins. This solution is then dialyzed against buffered divalent cation, e.g., calcium chloride, to produce a precipitate which can be called a DC (for direct calcium dialysis) cochleate.

(27) An additional, related method for reconstituting proteins or peptides into cochleates has been developed and is called the LC method (liposomes before cochleates). The initial steps involving addition of extracted protein or peptide, or carbohydrate, or DNA or combinations thereof, to dried down negatively charged lipid and cholesterol are the same as for the DC method. However, the solution is next dialyzed against buffer (e.g., 2 mMTES, 2 mML-histidine, 100 mMNaCl, pH 7.4) to form small liposomes containing the glycoproteins, peptides, DNA, and/or carbohydrates. A divalent cation, e.g., calcium, is then added either directly or by dialysis to form a precipitate which consists of protein- or peptide-cochleates.

(28) In the above procedures for making the cochleates of the present invention, the divalent cation can be any divalent cation that can induce the



formation of a cochleate or other insoluble lipid-antigen structures. Examples of suitable divalent cations include Ca.sup.++, Mg.sup.++, Ba.sup.++, and Zn.sup.++ or other elements capable of forming divalent ions or other structures having multiple positive charges capable of chelating and bridging negatively charged lipids.

(29) Protein- or peptide-cochleates can be lyophilized and stored at room temperature for indefinitely or can be stored in a divalent cation-containing buffer at 40.degree. C. for at least six months.

(30) After the protein- or peptide-cochleate precipitate is formed, the vaccine is made by diluting into an appropriate pharmaceutically acceptable carrier (e.g., a divalent cation-containing buffer).

(31) The reconstituted viral, bacterial, parasitic or animal proteins, peptides, carbohydrates, and/or DNA in the cochleates of the present invention can be employed as vaccines to render immunity to hosts treated with such compositions.

(32) Cochleate vaccines can include multiple synthetic peptide epitopes and thus offer a simple means of generating antiviral cell-mediated immunity in a genetically heterogeneous population. Formulations can be generated using mixtures of proteins or peptides either individually or as mixtures in various ratios.

(33) According to the method of the present invention, a host is immunized by administering an immunologically effective amount of the above-described protein- or peptide-cochleates. Advantageously, administration may be oral. However, the vaccine can also be administered by any of a variety of

[redacted]

art-recognized modes of administration, including intramuscular, subcutaneous, intradermal, intranasal, intra-ocular, intraperitoneal, intra-vaginal, intra-rectal and by lung aerosol. Appropriate dosages are determinable by, for example, dose-response experiments in laboratory animals or in clinical trials and taking into account body weight of the patient, absorption rate, half life, disease severity and the like. The number of doses, daily dosage and course of treatment may vary from individual to individual.

(34) Pharmaceutical formulations can be of solid form including tablets, capsules, pills, bulk or unit dose powders and granules or of liquid form including solutions, fluid emulsions, fluid suspensions, semisolids and the like. In addition to the active ingredient, the formulation would comprise suitable art-recognized diluents, carriers, fillers, binders, emulsifiers, surfactants, water-soluble vehicles, buffers, solubilizers and preservatives.

(35) . The skilled artisan can determine the most efficacious and therapeutic means for effecting treatment practicing the instant invention. Reference can also be made to any of numerous authorities and references including, for example, "Goodman & Gilman's, The Pharmaceutical Basis for Therapeutics", (6th Ed., Goodman, et al., eds., MacMillan Publ. Co., New York, 1980).

(36) The vaccines elicit humoral (antibody) and cell mediated (proliferation of helper T cells or cytotoxic "killer" activity by cytotoxic cells)--circulating and mucosal protective immune responses as shown in the examples below.

(37) EXAMPLES

(38) The present invention will now be described by means of specific examples which are not meant to limit the invention.

(39) EXAMPLE 1

(40) FORMATION OF PROTEIN-COCHLEATES USING SENDAI OR INFLUENZA VIRUSES

(41) Materials and Methods

(42) Materials. Bovine brain phosphatidylserine in chloroform was purchased from Avanti Polar Lipids, Birmingham, Ala. in glass ampules and stored under nitrogen at -20.degree. C. Cholesterol (porcine liver) grade I, .beta.-D-octyl-glucopyranoside, fluorescein isothiocyanate (FITC)-dextran (average mol. wt. 67,000), metrizamide grade I, and chemicals for buffers and protein and phosphate determinations, were obtained from Sigma Chemical Company, St. Louis, Mo. Organic solvents were purchased from Fisher Scientific Co., Fairlawn, N.J. Reagents for polyacrylamide gel electrophoresis were from BioRad Laboratories, Richmond, Calif. S1000 Sephacryl Superfine was obtained from Pharmacia, Piscataway, N.J. Thick walled polycarbonate centrifuge tubes (10 ml capacity) from Beckman Instruments, Palo Alto, Calif., were used for vesicle preparations, washes, and gradients. A bath type sonicator, Model G112SP1G, from Laboratory Supplies Company, Hicksville, N.Y. was used for sonications.

(43) Viral Growth and Purification. Virus was grown and purified essentially as described by M. C. Hsu et al., Virology, Vol. 95, page 476 (1979). Sendai (parainfluenza type I) and influenza (A/PR8/34) viruses were propagated in the allantoic sac of 10 or 11 day old embryonated chicken eggs. Eggs were inoculated with 1-100 egg infectious doses (10.sup.3 to

10.sup.5 viral
particles as determined by HA titer) in 0.1 ml of phosphate buffered saline
(0.2 gm/L KCl, 0.2 gm/L KH.sub.2 PO.sub.4, 8.0 gm/L NaCl, 1.14 gm/L Na.sub.2
H-PO.sub.4, 0.1 gm/L CaCl.sub.2, 0.1 gm/L MgCl.sub.2 6H.sub.2 O (pH 7.2)).
Eggs were incubated at 37.degree. C. for 48 to 72 hours, followed by
incubation at 4.degree. C. for 24 to 48 hours. Allantoic fluid was collected
and clarified at 2,000 rpm for 20 minutes at 5.degree. C. in a Damon IEC/PR-J
centrifuge. The supernatant was then centrifuged at 13,000 rpm for 60 minutes.
This and all subsequent centrifugations were performed in a Sorvall RC2-B
centrifuge at 5.degree. C. using a GG rotor. The pellets were resuspended in
phosphate buffered saline (pH 7.2) by vortexing and sonicating, followed by
centrifugation at 5,000 rpm for 20 minutes. The pellet was resuspended by
vortexing and sonicating, diluting, and centrifuging again at 5,000 rpm for 20
minutes. The two 5,000 rpm supernatants were combined and centrifuged at
13,000 rpm for 60 minutes. The resulting pellets were resuspended in
phosphate-buffered saline by vortexing and sonicating, aliquoted, and stored at
-70.degree. C. Sterile technique and materials were used throughout viral
inoculation, isolation, and purification.

(44) Extraction of Viral Glycoproteins and Lipids. Virus stored at
-70.degree. C. was thawed, transferred to sterile thick-walled polycarbonate
tubes, and diluted with buffer A (2 mMTES, 2 mML-histidine, 100 mM NaCl (pH
7.4)). It was pelleted at 30,000 rpm for 1 hour at 5.degree. C. in a Beckman
TY65 rotor. The supernatant was removed and the pellet resuspended to a
concentration of 2 mg viral protein per ml of extraction buffer (2M NaCl, 0.02M
sodium phosphate buffer (pH 7.4)) by vortexing and

[redacted]

sonicating. The nonionic detergent .beta.-D-octyl-glucopyranoside was then added to a concentration of 2% (w/v). The suspension was mixed, sonicated for 5 seconds, and placed in a 37.degree. C. water bath for 45 minutes. At 15, 30, and 45 minute incubation times, the suspension was removed briefly for mixing and sonication. Nucleocapsids were pelleted by centrifugation at 30,000 rpm for 45 minutes in a TY65 rotor. The resulting clear supernatant was removed and used in the formation of viral glycoprotein-containing cochleates. Some modification of the above procedure may have to be employed with other membrane proteins. Such modifications are well known to those skilled in the art.

(45) Formation of Cochleares

(46) A. Standard Cochleates

(47) Large, unilamellar, non protein-containing, phospholipid vesicles were made by a modification of the calcium-EDTA-chelation technique described by D. Papahadjopoulos et al., Biochem. Biophys. Acta, Vol. 394, page 483 (1975). Phosphatidylserine and cholesterol (9:1 wt ratio) were dried down in a clean glass tube under a stream of nitrogen. The lipid was resuspended in buffer A (pH 7.4) to a concentration of 6 .mu.Mol/ml by vortexing for 7 minutes. The resulting suspension of multilamellar vesicles was converted to small unilamellar vesicles by sonication under nitrogen at 5.degree.-10.degree. C for approximately 20 minutes in a bath-type sonicator. (Model G1125P16, Laboratory Supplies Co., Hicksville, N.Y.). These vesicles were dialyzed at room temperature against two changes of 250 ml of buffer A (pH 7.4) with 3 mM CaCl.sub.2. This results in the formation of an insoluble precipitate referred to as cochleate cylinders.

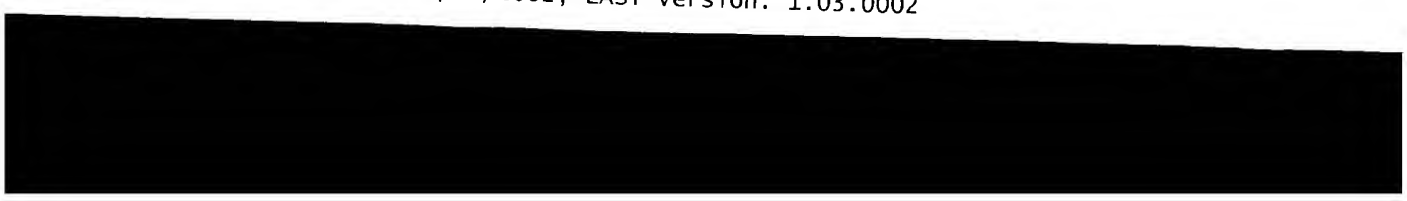
[redacted]

(48) B. DC Cochleates

(49) The envelope glycoproteins of Sendai virus account for about 33% of the total viral protein and are present in approximately equal weight to the viral lipid. An amount of phosphatidylserine and cholesterol (9:1 wt ratio) equal to 4 times the weight of the viral lipid was dried down under nitrogen in a clean glass tube. The amount of lipid added to the influenza virus extract was also equal to four times of the total viral protein. Supernatant from .beta.-D-octyl-glucopyranoside-extracted virus (see Extraction of Viral Glycoproteins and Lipids) was added, and the solution was vortexed for 5 minutes. The clear, colorless solution which resulted was dialyzed at room temperature against three changes (minimum 4 hours per change) of buffer A (2 mM TES N-Tris[hydroxymethyl]-methyl-2 aminoethane sulfonic acid, 2 mM L-histidine, 100 mM NaCl, pH 7.4) containing 3mMCaCl.sub.2. The final dialysis routinely used is 6 mM Ca.sup.2+, although 3 mM Ca.sup.2+ is sufficient and other concentrations may be compatible with cochleate formation. The ratio of dialyzate to buffer for each change was a minimum of 1:100. The resulting white calcium-phospholipid precipitates have been termed DC cochleates. When examined by light microscopy (.times.1000, phase contrast, oil), the suspension contains numerous spheres up to several microns in diameter with bumps or spikes on their surface, as well as needle-like structures.

(50) C. LC Cochleates

(51) Solubilized viral envelope was added to a film of phosphatidylserine and cholesterol (9:1 w/w) equal to four times the weight of the viral glycoproteins (which comprise one-third of the total protein of the



virus), and then
vortexed. This detergent solution containing solubilized
lipids and membrane
proteins was first dialyzed overnight using a maximum ratio
of 1:200 (v/v) of
dialysate to buffer A without divalent cations, followed by
three additional
changes of buffer leading to the formation of small protein
lipid vesicles.
These vesicles were converted to a protein- or
peptide-cochleate precipitate,
either by the direct addition of Ca.sup.2+ ions, or by
dialysis against one
change of buffer A containing 3 mM Ca.sup.2+ ions, followed
by one containing
buffer A with 6 mM Ca.sup.2+.

(52) EXAMPLE 2

(53) CIRCULATING ANTIBODY RESPONSES TO ORALLY DELIVERED
PROTEIN-COCHLEATE
VACCINES

(54) In order to make the vaccine, influenza virus was
grown, purified, and
the glycoproteins and lipids extracted and isolated as
described in Example 1.
Protein-cochleates were made according to the "LC
cochleate" procedure
described above.

(55) Cochleate vaccines containing the glycoproteins and
lipids from the
envelope of influenza virus and phosphatidylserine and
cholesterol were given
to mice by gradually dispensing 0.1 ml liquid into the
mouth and allowing it to
be comfortably swallowed. FIGS. 5(A) (from Experiment A)
and 5(B) (from
Experiment B) show resulting total circulating antibody
levels specific for
influenza glycoproteins, as determined by ELISA. Antibody
titer is defined as
the highest dilution that still gives the optimal density
of the negative
control.

(56) In Experiment A that generated the data shown in

FIG. 5(A), initial vaccine doses of 50, 25, 12.5 or 6.25 .mu.g of glycoproteins (groups 1 through 4 respectively) were administered at 0 and 3 weeks. The third and fourth immunizations (6 and 19 weeks) were at one fourth the dose used for the initial two immunizations. Bleed 1-Bleed 6 occurred at 0, 3, 6, 9, 19, and 21 weeks. The data demonstrate that high circulating antibody titers can be achieved by simply drinking cochleate vaccines containing vital glycoproteins. The response is boostable, increasing with repeated administration, and is directly related to the amount of glycoprotein in the vaccine.

(57) These observations were confirmed and extended in Experiment B that generated the data shown in FIG. 5(B). The dose range was expanded to include 100 .mu.g and 3.1 .mu.g initial doses. Vaccine was given at 0, 3 and 15 weeks, with the third immunization at one fourth the dose of the initial two. Bleed 1 to Bleed 6 occurred at 0, 3, 6, 15 and 16 weeks. Circulating influenza glycoprotein-specific responses were detectable after a single administration for the top five doses, and for all groups after two feedings. The data shown is for pooled sera from each group, but all mice given the four highest doses, and four of five mice in groups five and six, responded to the vaccine with circulating antibody titers ranging from 100 to 102,400. Group seven, which received no vaccine, had titers less than 50 for all mice at all time points.

(58) The antibody response is long lived. Titers 13 weeks after the third immunization (FIG. 5(A), bleed 5) and 12 weeks after the second immunization (FIG. 5(B), bleed 4) remained the same or within one dilution higher or lower than seen at 3 weeks after the previous boost.

(59) In Experiment C that generated the data shown in FIG. 6, a single oral dose of 50 .mu.g was administered. The mice were bled at 0, 28, 56 and 90 days and the antibody titer was determined by ELISA. The slowly increasing titers shown in FIG. 6 indicate the possibility of persistence and slow release of antigen from the cochleates.

(60) EXAMPLE 3

(61) PROTECTION FROM INTRANASAL CHALLENGE WITH LIVE INFLUENZA FOLLOWING ORAL IMMUNIZATION WITH GLYCOPROTEIN-COCHLEATES

(62) In order to determine whether oral administration of the subunit vaccine described in Example 2 could lead to protective immunity in the respiratory tract, the mice described in Experiment B of Example 2 were immunized with cochleates at 0, 3, and 15 weeks. The immunized mice were challenged by intranasal application of 2.5.times.10.sup.9 particles of influenza virus at 16 weeks. Three days after viral challenge, mice were sacrificed, and lungs and trachea were obtained. The entire lung or trachea was triturated and sonicated, and aliquots were injected into embryonated chicken eggs to allow amplification of any virus present. After three days at 37.degree. C., allantoic fluid was obtained from individual eggs, and hemagglutination (HA) titers were performed.

(63) Mice were also challenged with live influenza intranasally following oral cochleate administration in Experiment A of Example 2. Lungs were obtained three days later and cultured to detect presence of virus.

(64) The combined data for the two experiments is given in Table 1. These results are also shown graphically in FIG. 7.

(1) TABLE 1

Vaccine Protein	Trachea.sup.1 Total	Lungs.sup.2 Total	Lungs.sup.3 Total	Dose .mu.g	# Infected/ Mice from Experiment A	# Infected/ Mice from Experiment B.	# Infected/ .sup.1 Mice from Experiments A and B.
				100	0/5	0/5	
0/5	50	2/5	0/5	2/10	25	0/5	0/5
1/10	125	1/5	0/5	1/10	6.25	0/5	5/5
6/10	3.12	4/5	5/5	5/5	0	5/5	5/5
9/10							

.sup.2 Mice from Experiment B. .sup.3 Mice from

(65) The data in Table 1 shows that all five of the unvaccinated mice had sufficient virus in the trachea to infect the embryonated chicken eggs (greater than 10.sup.3 particles per trachea or at least one egg infectious dose (EID) per 0.1 ml of suspension). In contrast, the oral vaccine provided a high degree of protection from viral replication in the trachea. All mice in groups 1, 3 and 5 of Experiment B were negative for virus. Two mice in group 2, 1 in group 4, and 4 in group 6 (the lowest vaccine dose) of Experiment B had sufficient virus to test positive in this very sensitive assay used to detect presence of virus.

(66) The oral protein cochleate vaccine also provided protection against viral replication in the lungs. All twenty mice which received the four highest doses of vaccine were negative for virus when lung suspensions were cultured in embryonated chicken eggs (Table 1). All mice in the groups immunized with 6.25 .mu.g and 3.1 .mu.g glycoproteins and all mice in the unvaccinated control were positive for virus.

(67) Even in the lowest two vaccine doses, there was some inhibition of viral replication. When lung suspensions were diluted 1/10 and

inoculated into eggs, only one animal in the groups immunized with 6.25 .mu.g was positive, as compared to three in the groups immunized with 3.12 .mu.g and three in the unvaccinated control. Culturing of 1/100 dilutions resulted in one positive animal in each of the groups immunized with 6.25 and 3.12 .mu.g, but 3 of 5 remained positive in the unvaccinated group. In addition, for the two animals in the group that was immunized with 3.12 .mu.g, but which were negative at 1/100, only 50% of the eggs were infected at 1/10 and had low HA titers. In contrast, for the unvaccinated group, all eggs were infected and produced maximal amounts of virus at 1/10 and 1/100 dilutions.

(68) EXAMPLE 4

(69) ORAL ADMINISTRATION OF SENDAI COCHLEATE STIMULATES CIRCULATING ANTIBODY PRODUCTION

(70) C57BL6 mice were given cochleates containing Sendai virus glycoproteins orally at 0 and 3 weeks. They were bled at 0 (bleed 1), 3 (bleed 2), and 6 (bleed 3) weeks. Group 1 received approximately 50 .mu.g protein, Group 2 about 25 .mu.g, Group 3 about 12.5 .mu.g, Group 4 about 6.25 .mu.g, and Group 5 (negative control) received 0 .mu.g protein. The levels of Sendai specific antibodies in the serum pooled from 5 mice in each dose group were determined by ELISA. The results are shown in FIG. 8. It can be seen that strong antibody responses were generated, that the magnitude of the response was directly related to the immunizing dose, and that the magnitude of the response increased (boosted) after a second immunization.

(71) The response was extremely long-lived. FIG. 9 shows a breakdown of the classes and subtypes of Sendai-glycoprotein-specific

[redacted]

antibodies still
circulating 8 months later. The response is predominantly IgG, indicative of
the involvement in T cell help and establishment of long-term memory cells
associated with a secondary immune response. Surprisingly, the lowest dose
which initially had the lowest response, now had the highest circulating
antibody levels. This may be due to the immune system's down regulation of the
very high responses originally but allowing the low response to slowly climb.
This may also indicate a persistence and slow release of antigen. It is also
interesting and consistent with the use of the oral route of immunization that
significant IgA titers are generated and maintained (FIG. 10).

(72) EXAMPLE 5

(73) IMMUNIZATION WITH PROTEIN-COCHLEATES LEADS TO PRODUCTION OF
ANTIGEN-SPECIFIC LOCAL OR SECRETORY IgA

(74) Balb C mice were given Sendai glycoprotein-containing cochleates (50 .mu.g dose) by a single route or two routes simultaneously. They were boosted
using the same immunization protocol at 3 weeks. Saliva one was also 3 weeks
after the primary immunization. Saliva two was one week, and three was 3 weeks
after the second immunization. They were all boosted by oral administration at
24 weeks and saliva four was taken one week later. As can be seen in FIG. 11,
the oral route and oral plus IM routes generated the highest salivary IgA
titers. Demonstration of such high mucosal antibody titers following oral
immunization is of considerable significance and highly desired for protection
against organisms invading through mucosal surfaces.

(75) EXAMPLE 6

(76) PROLIFERATIVE RESPONSES ARE GENERATED TO ANTIGENS CONTAINED IN COCHLEATES

(77) Balb C mice were immunized three times by a variety of protocols with cochleates containing 50 .mu.g influenza glycoprotein at 0 and 3 weeks and with 12.5 .mu.g at 14 weeks. Some mice were sacrificed at 15 weeks and their spleens removed. (FIGS. 12(A) and (B), 13(A) and (B), 14(A) and (B), 15(A) and (B) and 16(A) and (B)). Part (B) of each figure shows the proliferative response to ultraviolet light-irradiated influenza virus over several days in culture. Part (A) shows the response to media as a control. Proliferative responses are measured for DNA synthesis by .sup.3 H-Thd uptake into cells. All routes led to antigen-specific proliferation. Oral primary followed by 2 IM boosts gave the highest response, with 3 oral immunizations were a close second.

(78) EXAMPLE 7

(79) CYTOLYTIC ACTIVITY IS GENERATED UPON IMMUNIZATION WITH SENDAI COCHLEATES

(80) A 50 .mu.g protein dose of Sendai glycoprotein-containing cochleates was given orally. Two weeks later the animal (Balb/C mouse) was sacrificed and spleen cells obtained. Cytolytic activity of the spleen cells was measured by their ability to cause the release of Chromium 51 from target cells presenting Sendai antigens. The non-immunized mouse did not kill Sendai virus (SV) pulsed cells with in culture restimulation (N/SV/SV) or non-Sendai presenting cells (N/N/N). (FIG. 17). In contrast, Sendai cochleate immunized mice killed SV pulsed targets to a very high degree and non-pulsed targets to a lesser degree. Cytolytic activity is crucial to clearance of cells

[redacted]

infected with viruses, or intracellular parasites or to cancer cells. It is a highly desirable activity for a vaccine to induce, but classically has not been seen with most non-living vaccines. This is an important feature of protein-cochleate vaccines.

(81) EXAMPLE 8

(82) PEPTIDE COCHLEATE VACCINES GIVEN ORALLY GENERATE ANTIBODY RESPONSES

(83) Cochleates containing a peptide from the surface glycoprotein of the AIDS virus cross-linked to phosphatidylethanolimine were given to mice orally three times (0, 3 and 6 weeks). (FIG. 18). In addition, cochleates containing only Sendai glycoproteins or Sendai plus the HIV peptide (amino acids 494-518) were given to separate groups of mice. Serum antibody levels were determined by ELISA. When 494-518 was formulated alone, significant antibody titers were not seen. However, with Sendai a titer of 1000 was obtained to the peptide and 2000 to Sendai. The ability to stimulate circulating antibody responses to a peptide given orally represents a significant achievement for this new class of vaccines.

(84) EXAMPLE 9

(85) ORAL IMMUNIZATION WITH SENDAI-COCHLEATES STIMULATES MUCOSAL CELL MEDIATED RESPONSES

(86) Balb C mice were given cochleates containing 50 .mu.g of Sendai glycoproteins orally and intraperitoneally simultaneously. They were sacrificed 2 weeks later, and Peyer's Patches were obtained by cutting from the surface of the small intestine. Cells isolated from the Peyer's Patches were incubated in culture with ultraviolet light-inactivated

[redacted]

Sendai virus as a stimulatory antigen. Proliferation was measured as .sup.3 H-Thd uptake. It can be seen that while cells from a naive (unimmunized) mouse proliferate to some degree in response to Sendai virus, the immunized animal proliferated to a much greater degree. This indicates that the Sendai cochleares survived the stomach to be taken up by the microfold (M) cells of the small intestine and stimulated the T helper cells present there. The ability to do this is crucial to a successful oral vaccine.

(87) While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

CLAIMS:

What is claimed is:

1. A method of immunizing a host, comprising administering a biologically effective amount of a protein- or peptide-cochleate which comprises the following components:

a) a protein or peptide component to which an immune response is elicited,

b) a negatively charged lipid component, and

c) a divalent cation component.

2. The method of claim 1, wherein the component a) is a peptide.

3. The method of claim 2, wherein the peptide is hydrophobic.

4. The method of claim 2, wherein the peptide is covalently linked to a

phospholipid.

5. The method of claim 1, wherein the component a) is glycoprotein.

6. The method of claim 1, wherein the component a) is membrane protein.

7. The method of claim 1, wherein the component a) is membrane glycoprotein.

8. The method of claim 1, wherein the protein or peptide component is from a bacterial or animal virus.

9. The method of claim 8, wherein the protein component is membrane glycoprotein from Sendai virus.

10. The method of claim 8, wherein the protein component is membrane glycoprotein from influenza virus.

11. The method of claim 1, wherein the protein or peptide component is from a bacterium.

12. The method of claim 1, wherein the protein or peptide component is from a parasite.

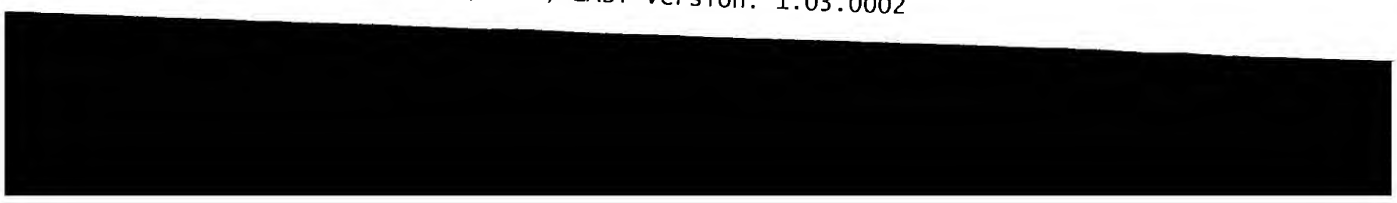
13. The method of claim 1, wherein the protein or peptide component is from an animal cell.

14. The method of claim 13, wherein the animal cell is from a mammal.

15. The method of claim 14, wherein the mammal is a human being.

16. The method of claim 1, wherein the protein or peptide component is from an animal tissue.

17. The method of claim 16, wherein the animal tissue is from a mammal.



18. The method of claim 17, wherein the mammal is a human being.

19. The method of claim 1, wherein the negatively charged lipid component is phospholipid.

20. The method of claim 1, wherein the phospholipid is selected from the group consisting of phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, and phosphatidic acid.

21. The method of claim 1, wherein the divalent cation component is a cationic compound capable of chelating and complexing negatively charged lipids.

22. The method of claim 21, wherein the divalent cation component is selected from the group consisting of Ca.sup.++, Mg.sup.++, Ba.sup.++ and Zn.sup.++.

23. The method of claim 22, wherein the divalent cation component is Ca.sup.++.

24. The method of claim 1, wherein said administering is by a peroral route.

25. The method of claim 1, wherein said administering is by an intramuscular, a subcutaneous, an intradermal, an intranasal, an intra-ocular, an intraperitoneal, an intra-vaginal, an intra-rectal or a lung aerosol route.

26. The method of claim 22, wherein the divalent cation component is Ca.sup.++ or Mg.sup.++.

[redacted]